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VOL. XXX. No. 1.

THE EARLY DEVELOPMENT OF ASPLANCHNA HERRICKII DE GUERNE.

A CONTRIBUTION TO DEVELOPMENTAL MECHANICS.

BY HERBERT S. JENNINGS.

WITH TEN PLATES.

CAMBRIDGE, MASS., U. S. A :
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No. 1. — *The Early Development of Asplanchna Herrickii de Guerne. A Contribution to Developmental Mechanics.*[1] By HERBERT S. JENNINGS.

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INTRODUCTION.

The following pages contain a study of the early development of an organism, with especial reference to recent theories in regard to the laws of cleavage and the relation of cleavage to morphogenesis.

Many theories and so called laws have been set forth concerning the factors determining the manner and rate of cleavage. These have taken the form chiefly of theories in regard to the causes of the *direction of the spindle*, of the *equality* or *inequality* in size of the products of division, and of the *relative rapidity* with which the different cleavage cells divide. Yet few attempts have been made to interpret consistently the cleavage of any given organism with relation to any or all of these theories. The sketch of Braem ('94) with regard to the Echinoderm egg, and the recent studies of Ziegler ('95) and zur Strassen ('96)[1] on the Nematode egg, are almost the only works that can be cited in which an attempt has been made to show the relation of any theory or theories to the series of normal cleavages in any animal. In other discussions the theories have been based upon experimental evidence or upon scattered observations. Yet it is, of course, the *normal processes* for which explanations are desired; scattered observations may be adduced for almost any view. It seems of the greatest importance, therefore, to show clearly the exact relation which the theories hitherto proposed have to the actual series of cell divisions in the development of particular organisms.

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[1] In view of the close similarity of some of my conclusions with some of those in the more recent ('96) of two papers by zur Strassen, it may be proper to state that a copy of the present paper, exactly as here published, with the exception of some verbal alterations and the addition of a few references, was deposited with the Faculty of Arts and Sciences of Harvard University on April 30, 1896, while zur Strassen's ('96) paper was not received here till May 13.

Furthermore, there is much discussion of the question as to whether cleavage is a mere quantitative separation of a single mass into smaller masses similar in nature to each other and to the original egg, or whether it is accompanied by a differentiation of the separated blastomeres, — as a result either of qualitative division or other changes.

A third question of theoretical interest, somewhat related to the last, is whether the method of cleavage has a direct mechanical relation to future morphogenetic processes, or whether it is merely the passing of partitions through a mass of protoplasm, the order in which this occurs and the arrangement of the partitions being immaterial. For example, Is gastrulation a process independent of cleavage and merely requiring the latter as a prerequisite, — as the planting of seeds must be preceded by ploughing, — or is gastrulation in some way connected with or dependent upon the manner of cleavage? Stated in the most general terms, this is the question: Is cell division a *direct* morphogenetic factor, or are the real formative processes dependent upon the introduction of other factors after the cleavage is finished?

With these questions in mind, I have studied the development of an organism of the class Rotifera throughout those stages of development in which it is possible to make the *cells* the units of observation, — that is, through cleavage and gastrulation and somewhat later.

Broadly stated, the object of the work may be expressed as *the analysis of the early development of an organism into the simplest factors possible.*

The development of Asplanchna Herrickii has not been studied previously, and in the course of this paper it will be necessary to discuss some matters which are of importance primarily to persons who are engaged particularly with the morphology of the Rotifera, and which are not of especial interest from a morphogenetic standpoint. In order to distinguish these two lines of discussion, I shall divide the work into two main portions. Part First will contain all matters bearing upon developmental mechanics. Here will be found the minute description of the cleavage, gastrulation, and other processes, as well as a discussion of their bearing upon the problems of morphogenesis. Part Second will contain a brief review of previous knowledge of the organism studied, a comparison of the development, so far as traced, with the development of other Rotifera, and a discussion of some of the conditions described by other authors. These principal parts will be followed by a third, on material, methods, and other subordinate matters, and the whole will be closed by a summary of the more important conclusions arrived at.

PART FIRST. — DEVELOPMENTAL MECHANICS.

I. Statement of Problems.

We shall deal in the following pages with (1) cleavage, (2) gastrulation, and (3) the relation of these to each other.

1. CLEAVAGE.

It will be necessary, in studying the cleavage and the factors determining it, to enter into minute details as to the movements of asters, the form and dimensions of cells, and other similar matters; the effort of following this, in itself somewhat laborious, description will be much lightened by holding in mind the problems upon which it bears. I shall therefore give first a statement of the main theories which have been advanced as to the determining factors in cell division.

Cell division presents three aspects, in each of which its nature is in some way determined. (A) As to the *direction* of cleavage: the position in which the new septum is to appear. Since this bears a definite relation, in general, to the position of the spindle leading to the cleavage, we may speak of this aspect as the determination of the direction of the spindle. (B) As to the *relative size* of the two products; whether the division is equal or unequal. (C) As to the relative *time* of division, or the interval between successive cleavages.

Besides these, we have (D) the question of the *qualitative* nature of cleavage. Are all the cells that are produced of similar structural and material character, or is cleavage accompanied by qualitative differentiation of the blastomeres, — either as a result of qualitative karyokinesis or otherwise?

A. *Theories as to the Factors determining the Direction of the Spindle and the Position of the new Cell Wall.*

The theories as to the factors determining the direction of cleavage are numerous, and have been much discussed of late. General reviews of these theories will be found in Driesch ('92, p. 26), Braem ('94, p. 340), Ziegler ('94, p. 136), and McMurrich ('95). I shall give here as brief and precise a statement of each theory as possible, first in my own words, then, so far as practicable, in a quotation from the author.

(1) *Berthold's principle of least surfaces.* — Berthold ('86) holds that the form and relative position of cells, and as a consequence their direc-

tion of cleavage, is determined, partially at least, by the same factors which determine the form and relative position of soap bubbles in a mass. As a result of surface tension, the cells take such forms as to occupy the given space with the least possible surface areas. New septa will appear in such positions that their surfaces will be the least possible areas that could divide the cell into parts of the required size. "Die Lamellensysteme ordnen sich so an, die einzelnen Lamellen krümmen sich in der Weise, dass die Summe der Oberflächen aller unter den gegeben Verhältnissen ein Minimum wird." (Berthold, '86, pp. 219, 220.)

(2) *Hertwig's law of the spindle in the longest axis of the protoplasmic mass.* — According to Hertwig's well known view, as a result of the interaction of nucleus and protoplasm, the spindle during division comes to lie in such a position that its longitudinal axis coincides with the axis which passes through the greatest protoplasmic mass. "Es lässt sich hier das zweite allgemeine Gesetz aufstellen, dass die beiden Pole der Theilungsfigur in die Richtung der grössten Protoplasmamassen zu liegen kommen, etwa in derselben Weise, wie die Lage der Pole eines Magneten durch Eisentheile in seiner Umgebung beeinflusst wird." (Hertwig, '93, p. 175.)

(3) *Braem's theory of separation in the direction of the greatest space for development.* — This is a modification of the principle of least pressure, first enunciated by Pflüger ('84). Since Pflüger's principle, considered from a purely mechanical standpoint, seems irreconcilable with the nature of the material on which it was supposed to act, and since Braem's view is based on an essentially different conception of the nature of the phenomena, I have not thought it necessary to take into direct consideration Pflüger's view.

Braem holds that when an egg is subjected to unequal pressure, the spindle places itself in such a position that the resulting products shall have the freest opportunity for development; that is, in the direction of least resistance. The rule is not the expression of a purely mechanical force, but is to a certain extent teleological in character. "Die Spindel eines ungleichem Druck unterliegenden Eies stellt sich in derjenigen Richtung ein, in welcher der räumlichen Entfaltung der Zelle und ihrer Teilprodukte der freieste Spielraum geboten ist. Ich glaube, dass diese Fassung trotz oder vielmehr gerade wegen ihres teleologischen Gehaltes dem Wesen der Sache besser entspricht als die rein mechanische Deutung." (Braem, '94, pp. 341, 342.)

The result is held to be due to a sort of sensory power resident in the egg, "eine Art Tastsinn, durch den es der Zelle möglich wird, sich über ihre unmittelbare Umgebung zu orientieren und demgemäss einzurichten." (p. 342.)

(4) *Roux's theory of a compromise between the tendency immanent in the nucleus and the tendency due to the form of the protoplasmic mass.* — Roux holds that the spindle places itself in one of the positions of stable equilibrium in relation to the protoplasmic mass, — therefore, at least generally, in the longest axis of the protoplasmic mass, though sometimes at right angles to that axis, the factor that decides which of these positions shall be taken being an immanent tendency in the nucleus to divide in a certain direction.

"Richtiger ist es zu sagen: Die Kernspindel der Furchungszellen stellt sich in die, resp. in eine Richtung festesten Gleichgewichtes der tractiven Einzelwirkungen der Protoplasmamasse. Diese Richtung entspricht überwiegend häufig annähernd oder ganz der grössten durch den Mittelpunkt der Protoplasmamasse gehenden Dimension.

"Diese Richtung des Gleichgewichtes wird aber nicht vollkommen vom Protoplasma allein bestimmt, sondern sie kann, wie ich bereits 1884 und 1885 auf Grund von Experimenten erschlossen habe, von der Lage der immanenten Teilungsrichtung des Kernes zu den Hauptrichtungen des Protoplasmakörpers abhängig sein; denn ich erhielt bei symmetrisch gestalteten 'linsenförmig' deformirten, mit den grössten Fläche senkrecht stehenden Froscheiern *zwei* Prädictionsrichtungen der Spindeleinstellung: die Richtung der grössten und der kleinsten durch den Massenmittelpunkt gehenden Dimension, erstere allerdings wieder die überwiegend häufige." (Roux, '94, p. 152.)

It is to be noted that this theory does not attempt to give any rule by which the position of the spindle is necessarily determined; the tendency of the nucleus is simply "immanent," and its factors unknown.

In addition to these four well characterized theories, a number of less definite or partial views have been set forth, — some proposing factors which may influence, though not alone determine, the position of the spindle. A number of the more important of these will be mentioned.

(5) *Heidenhain's problem of a definite angle of rotation* ("Problem der gesetzmässigen Drehungswinkel"). — Heidenhain ('94, p. 719) thinks it probable, or at least possible, that careful investigation will show that in a given tissue the position which the spindle takes at the time of division is a result of its rotation through a definite angle, determinable for the given tissue, after the first formation of the spindle by the separation of the asters. This separation of the asters is held

to be at first in a line at right angles to the axis of the preceding spindle; then, by a rotation through an angle characteristic for the tissue, the definitive position is reached. The position which the spindle is finally to take is therefore determined at the time the asters separate. "Soweit ich indessen die Lage übersehen kann, ist die schliessliche Stellung der Spindel von dem Moment an fest gegeben, in welchem die Theilung des Muttermikrocentrums stattfand." (Heidenhain, '95, pp. 555, 556.)

(6) *Sachs's view, that the walls separating the cells meet one another at right angles.* — This (Sachs, '78, p. 1070) can hardly be considered as more than a statement of a condition commonly found. Berthold ('86, p. 252) and Hertwig ('93, p. 177) have endeavored to show that the condition is explainable as a result of the theories proposed by them.

(7) *Rauber* ('83, p. 276) *holds that there is evidence that the asters of the different blastomeres exercise an attraction for each other in such a way that, in a given area composed of a number of cells, the spindles must take such positions as to bring about a condition of equilibrium among the asters.* — "Beurtheilt man die Verschiedenheiten der Furchennetzes von der Stellungen der karyokinetischen Achsen aus, so gewinnt es den Anschein, als ob die neu entstehenden Centren eines Blastomers auf diejenigen der angrenzenden Blastomeren einzuwirken vermögen und die Richtung ihrer Achsen beeinflussen." (Rauber, '83, p. 280.)

(8) *Braem's principle of equal resistance at the two ends of the spindle.* — Subordinate to his principle of least resistance, Braem holds that the spindle tends to take such a position that the pressure at the two ends is the same. "Es ist das Princip des *gleichen* Widerstandes, wodurch die horizontale Lage der Spindel bedingt wird. Wir müssen annehmen, dass der Kern von vornherein das Bestreben hat, sich gleichmässig nach beiden Seiten hin auszudehnen und somit auf eine äquale Zellteilung hinzuwirken." (Braem, '94, p. 345.)

In the following description these theories will be kept in mind, and the bearing of the observations upon them pointed out. It will appear that, for certain of the theories, the conditions in the egg of Asplanchna present crucial tests.

B. *Equality or Inequality of Cleavage.*

The second aspect under which cleavage is determined is with regard to the *relative size* of the two products. What is it that determines whether the division shall be equal or unequal?

Concerning the factors which determine the equality or inequality of cleavage, two theories have been proposed. According to the view which is perhaps that most generally known, the cause of unequal cleavage lies in the relative distribution of yolk material and formative protoplasm. The interaction between nucleus and cell contents, which determines the position of the dividing nucleus, exists only between the nucleus and the formative protoplasm, not between the nucleus and the yolk material. As a consequence of this interaction, the nucleus tends to take a position in the centre of the mass of *formative protoplasm.* When one region of the cell is composed largely of yolk material, in a mere meshwork of protoplasm, while another region is made up entirely of protoplasm, the dividing nucleus must separate equal masses of formative protoplasm, and thus may divide the *entire* mass into very unequal parts, — one containing a certain mass of protoplasm only, the other an equal mass of protoplasm and a large additional mass of yolk material. The theory has recently been formulated by Hertwig as follows: "Die Folge dieser Wechselwirkung aber ist, dass der Kern stets die Mitte seiner Wirkungssphäre einzunehmen sucht. . . . Wechselwirkungen finden zwischen dem Kern und dem Protoplasma, nicht aber zwischen ihm und dem Dottermaterial statt, welches bei allen Theilungsprocessen sich wie eine passive Masse verhält. Ungleichmässigkeiten in den Protoplasmavertheiluug müssen sich daher auch auf Grund des obigen Satzes in der Lage des Kerns geltend machen, und zwar muss derselbe nach den Orten der grösseren Protoplasmaansammlung hinrücken." (Hertwig, '93, pp. 172 and 174.)

Braem's principle of equal resistance at both ends of the spindle is in character related to this view of Hertwig. Besides the effect of it in determining the direction of the spindle, this supposed principle is likewise of effect in determining the equality or inequality of cleavage, as appears from the quotation from Braem given on page 7.

C. *Determination of the Time of Division, or the Interval between Successive Cleavages.*

The same factor which is held to determine the relative size of the cells was also held by Balfour, with whom Hertwig agrees (Hertwig, '93, p. 180), to determine the relative rapidity of cleavage. The greatest interval between successive cleavages is found in cells which contain the greatest amount of yolk relative to the amount of contained protoplasm. "The rapidity with which any part of an ovum segments varies *ceteris paribus* with the relative amount of protoplasm it contains; and the size of the segments formed varies inversely to the relative amount of the protoplasm." (Balfour, '80, p. 99.)

D. *Differentiation during Cleavage.*

Besides these questions in regard to the form and rate of cleavage, we have also the question of the *qualitative nature of cleavage.* Is cleavage merely a quantitative process, or is it accompanied by a differentiation of the separated cells? And if the latter is the case, by what means is this differentiation accomplished?

The view once maintained, that cleavage is entirely unaccompanied by differentiation of the separated cells, may be said to be nearly or entirely given up; the questions which remain relate to the means by which this differentiation is brought about. In regard to this several well defined views exist.

1. Roux holds that the differentiation accompanying cleavage is a result of qualitative karyokinesis; i. e. at a given cell division the two products receive *nuclear* material of different nature.

2. Driesch maintains that the differentiation which may accompany cleavage is due to the specific *cytoplasmic* structure of the egg, different parts of the egg being of different constitution, so that when this differentiated mass is separated into parts, these parts receive different sorts of cytoplasm. That is, the qualitative division is in the cytoplasm, not in the nuclear material. "Ich habe schon oben gesagt, dass ich ein Verschiedenwerden der Furchungszellen während der Furchung gern zugebe, aber hierin nichts anderes als die Folge eines spezifischen Plasmabaus des Eies sehe." (Driesch, '94, p. 100.)

3. According to Wilson and Hertwig the differentiation accompanying cleavage is due, largely at least, to the interaction of the blastomeres, after division has taken place. This does not exclude the possibility of the existence at the same time of a qualitative division of the cytoplasm, as stated above (2).

2. LATER DEVELOPMENTAL PROCESSES.

With regard to the later developmental processes, it will not be necessary to give here a review of the various factors and theories which have been set forth by different authors. Driesch ('94) gives an extended analysis of the morphogenetic process and its factors, and Davenport ('95) presents a detailed list of the different processes concerned in development. It is sufficient here to propose a single question: What is the relation of the cleavage process to the secondary morphogenetic processes? Driesch's well known experiments indicate that, in the case of the sea-urchin, the manner of cleavage is entirely unimportant for the later morphogenetic processes. Gastrulation, for example, occurs in the same manner, after the most varied and fundamental alterations of the cleavage. Is this a fact which is capable of generalization, — of application to different animals and different methods of gastrulation? Doubtless the only positive answer to this question must come from experimental studies; but a careful descriptive analysis of the process in Asplanchna gives results which, if the egg were a mechanism of the ordinary physical sort, would be definite and conclusive.

II. Descriptive Portion.

1. Form and Structure of the Egg.

The development of the embryo in Asplanchna Herrickii takes place within the body of the mother, the egg lying enclosed in the enlarged oviduct, close to the ovary. The chief axis of the developing embryo bears no relation to the position of surrounding organs of the mother, the egg lying in the oviduct as it might within a protecting sac of any foreign material, its position determined by chance circumstances. In cases where two embryos are present, their axes may make any angle with each other.

For study it is necessary to dissect out the eggs. A full account of the methods of work is given in Part Third; here it is important to note two facts: (1) All the work was done on preserved material; (2) Each egg comes from a different individual, and is therefore in at least a slightly different stage from every other. A considerable number of eggs showing any given process, as, for example, the first cleavage, gives therefore a series of stages, so that a complete idea of the changes taking place may be gained.

The unsegmented egg is approximately an ellipsoid of nearly equal axes, one end often slightly more pointed than the other. The form and proportions vary a little, as do also the absolute dimensions. In many eggs it is difficult to distinguish a more pointed end. The proportion of the longer to the shorter axis is about as 9 to 8, and the average dimensions of the egg are about 90 μ through the longer axis by 80 μ through the shorter. Variations from a minimum of 84 μ by 70 μ to a maximum of 97 μ by 83 μ were observed.

Whether an egg membrane is present or not is exceedingly difficult to decide; and I have not succeeded in thoroughly satisfying myself upon that point. The walls of the oviduct invest the egg closely, and generally cannot be removed, so that a thin membrane, if present, could not be detected. In most cases where it was possible to remove the walls of the oviduct entirely, no membrane could be seen either in sections or whole preparations, the egg appearing to be naked. In a few cases, however, in which cleavage had recently taken place and the cleavage furrows were marked, it could be observed that the smooth outline of the egg was preserved even above the cleavage furrow, either by means of a membrane continuing across the furrow, or, what seemed from the appearance of the preparations more probable, owing to the presence in the furrows of a fluid mass, perhaps exuded from the egg. Such a case is shown in Plate **1**, Fig. 4. On other grounds, however, it seems possible that an extremely delicate membrane is present. Lameere ('90) states that in Asplanchna Sieboldii, which is likewise viviparous, it was possible to observe definitely a very delicate membrane surrounding the egg, especially at the time of the formation of the polar cell.

This question of the presence or absence of an egg membrane is of importance from a mechanical standpoint, owing to its bearing upon the question as to what preserves the ellipsoidal form of the egg. The form is retained throughout all the early developmental processes; cleaving cells do not project above the general surface of the egg, nor do the products of cleavage become spherical, touching at a few points only, as is common in the Mollusca and other groups. This retention of the ellipsoidal shape by the egg compels the cleaving cells to take various peculiar forms, which allow of a direct test of some of the theories of cell division above stated. It is also a most important factor in the process of gastrulation, so that it becomes of great interest to discover what it is that preserves this form.

It is evident that surface tension would tend to produce a spherical rather than an ellipsoidal form. Roux ('95) has recently proved that blastomeres have a direct attraction for each other; but an equal attraction throughout the mass would produce a spherical form, and an unequal attraction, such as would produce a regularly ellipsoidal form, is very difficult to conceive of, especially as this attraction would have to vary regularly with the shifting of the contents of the egg. A membrane of equal elasticity in all parts would likewise result in the production of a spherical form. The only direct mechanical factor that seems capable of explaining the continued ellipsoidal form is the presence of a non-elastic membrane of the exact size and shape of the egg. But during the later development the embryo enlarges and changes its form;

the membrane cannot therefore be absolutely inelastic, but might be of such strength as to act as an inelastic membrane with regard to such slight forces as are exerted within the egg during its early development. The existence of a membrane of this peculiar character is, however, very improbable, and it loses all its explaining power when the egg of another rotifer, Melicerta ringens, is taken into consideration. In this species the egg is not a regular ellipsoid or oval, but is of an irregular shape, one side being curved in profile, the other straight. (See the figures of Zelinka, '91.) This form is retained during development exactly as in Asplanchna, yet is not explainable on the assumption of a membrane. This question is discussed more fully later.

The cytoplasm of the egg is closely filled with fine yolk granules. These are distributed uniformly throughout the egg (except that they are not present in the asters), so that there is no visible differentiation into regions containing greater and less amounts of yolk material.

The development of Asplanchna priodonta Gosse was also examined for comparison with that of Asplanchna Herrickii. The egg of this species is similar throughout to that of Asplanchna Herrickii, save that it is smaller. The average dimensions are about $70\,\mu$ by $60\,\mu$. The egg of Asplanchna priodonta is shown in Figure 29, Plate **4**, drawn to the same scale as the figures from Asplanchna Herrickii.

2. Maturation.

The formation of the polar cell in Asplanchna Sieboldii has been described by Lameere ('90) from observations upon the living egg. The general features of the process are similar in Asplanchna Herrickii, though the finer nuclear phenomena differ from those described by Lameere. An account of the finer nuclear phenomena is, however, foreign to the purpose of this paper: it is necessary to describe merely the general features of the process, especially concerning the place where the process occurs, in relation to the later orientation of the embryo.

As is now well known, but a single polar cell is commonly formed in the parthenogenetically developing eggs of the Rotifera. The subject has received full discussion, especially by Weismann und Ischikawa ('87) and Lameere ('90). It may be noted that Zelinka ('91) observed that in a number of eggs of Callidina two polar cells were formed; whether these arose by division of a single one, or whether the two were formed separately from the egg, is not stated. In no case have I obtained any evidence indicating the formation of more than a single polar cell in Asplanchna.

In immature eggs the large germinative vesicle is commonly found in an eccentric position, with no very apparent relation to the axes of the egg. In Asplanchna Sieboldii, according to Lameere ('90), just before the movement of the germinative vesicle toward the spot where the polar cell is formed, it lies in the long axis of the egg nearer one pole, — in the position where later the first cleavage spindle is located. This is probably the case also in Asplanchna Herrickii; but before the germinative vesicle has begun to show the changes indicating the formation of the maturation spindle it is difficult in preserved material to get evidence as to the proper sequence of the stages observed.

Just before the maturation spindle is formed, the nucleus moves toward the periphery of the egg, and begins to lose its spherical shape. It takes a position close to the surface, not at the equator, but nearer one of the poles of the egg, as shown in Figure 1. In cases where the differentiation into a more pointed pole and a blunter one is visible, the nucleus always lies nearer the more pointed pole. Here a spindle is formed, and the maturation division takes place. The polar cell thus formed does not lie upon the outer surface of the egg as a free body, but from the first is pressed into the substance of the yolk (Fig. 2), as if by a firm membrane, in the manner described by Lameere for Asplanchna Sieboldii. The nucleus begins to withdraw from the periphery, at the same time resuming the spherical form, leaving the polar cell a flattened, disk-like body, not projecting above the general surface of the egg. This condition of the egg is shown in Figure 2.

From the first, therefore, the polar cell is imbedded in the substance of the egg, so that it cannot suffer displacement during the processes which follow. As will be shown in the course of this paper, the place where the polar cell is formed marks the point on the surface of the egg opposite to that at which gastrulation takes place. This is contrary to the statement made by Zelinka ('91) for the egg of Callidina, and contrary to his general statement for the Rotifera as to the relation of the place of polar cell formation to the later axes of the embryo. As this matter is not of especial interest from the standpoint of developmental mechanics, a full discussion of the difference between my account and that of Zelinka is reserved for Part Second. There it will be shown by evidence from Zelinka's own work, as well as that presented here, that his general statement of the relation of the place where the polar cell is formed to the axes of the egg in the Rotifera cannot be considered true for other forms than Callidina russeola, whatever may be the conditions in that species.

3. Orientation of the Developing Embryo.

The first cleavage plane is transverse to the long axis of the egg, and divides it into two unequal parts (Figure 6). The plane passes through the place where the polar cell was formed; the smaller cell includes, therefore, that end of the egg nearest to which the polar cell is located. As previously stated, this is also the more pointed end of the egg, when any difference in the two ends is distinguishable.

The second cleavage is approximately at right angles to the first, and nearly in the long axis of the egg. It also passes through the region where the polar cell was formed.

As previously stated, the oval or ellipsoidal form of the egg is retained throughout the early development. This form is independent ot the precise arrangement of the material of which the egg is composed. It is as if the egg substance were enclosed in a rigid mould of oval or ellipsoidal form. Within this mould the (fluid?) contents may shift their position widely, without influencing the form of the mould. Thus, at the first cleavage, the material of the smaller blastomere occupies all of one end of the egg. In the ten-cell stage (Plate **3**, Figs. 20–25) the same *form* is still preserved as if in a rigid cast, but the *material* which previously formed the smaller of the first two blastomeres has shifted from the end to one side of the egg. It therefore is necessary to have some term by which to designate the two ends of this constant *form*, as distinguished from the shifting blastomeres themselves. I shall henceforth speak of that end of the egg at which lies the smaller cell in the two-cell stage as the *micromere* end of the egg, while the opposite region, where the larger blastomere lies, will be called the *macromere* end. These terms refer to the *form* of the egg, without regard to the shifting contents.

The orientation which I shall adopt for the egg itself is similar to that used by Wilson, Heymons, Conklin, Lillie, Kofoid, and other recent workers on cell lineage. The region where the polar cell is formed, and which afterward lies opposite the blastopore, will be called the *animal pole;* it marks the *dorsal surface.* The opposite point is the *vegetative pole*, marking the *ventral surface,* — the position of the future blastopore. *Dorsad* signifies always toward the animal pole, or place where the polar cell was formed; *ventrad,* in the opposite direction, toward the region where the blastopore is found at a late stage. The orientation is thus based upon the structure of the *gastrula.* The *chief axis* of the gastrula is the line connecting the animal and vegetative poles.

The first cleavage plane, though coinciding with the chief axis of the *gastrula,* is, as shown by the later development, transverse to the long axis of the *embryo.* The smaller cell of the two-cell stage is *anterior,* since its products occupy the anterior margin of the blastopore; the larger cell is *posterior,* its products forming the posterior lip of the blastopore. The second cleavage plane, though modified in the posterior part of the egg, is approximately longitudinal. In the four-cell stage (Plate **2**, Fig. 8) the two cells A^3 and B^3, resulting from the division of the smaller cell, $\overline{AB}^2$, are respectively left anterior and right anterior, while C^3 and D^3, produced by the division of the larger cell, $\overline{CD}^2$, are respectively right and left posterior.

A section taken transversely to the chief axis of the gastrula will be spoken of as a *transverse section.* A section at right angles to this, passing from anterior to posterior and including the animal and vegetative poles, is a *sagittal* section. A section at right angles to both of these, cutting both the animal and the vegetative pole and passing through the right and left sides, is a *frontal* section.

As will be seen from the above, in the two-cell and four-cell stages, the micromere end coincides with the anterior, the macromere end with the posterior end.[1]

The orientation given above is based upon the relation of the egg to the axes of the gastrula; the same is true of the orientation used in most of the recent works upon cell lineage. It differs fundamentally from the orientation used by Zelinka ('91) for the developing egg of the rotifer Callidina russeola. In that species the egg is of the same form as in Asplanchna. After extensive shifting during development, the anterior end (in both Asplanchna and Callidina) comes to lie in the region of that end of the egg which I have called the macromere end. Zelinka calls this end of the egg, therefore, the anterior end, the opposite (my micromere end) the posterior end. Anterior and posterior in Zelinka's orientation of course remain constant with regard to the *form of the egg,* but not with relation to the *parts of the embryo.* Thus, if

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[1] It is of the greatest importance to observe that I do not use the terms "micromere end" and "macromere end" in the same sense in which "micromere pole" and "macromere pole" are sometimes used, as synonymous with "animal pole" and "vegetative pole." The two terms are used only as a convenient way of indicating a *peculiarity* of the rotifer egg.

Zelinka's orientation were adopted, the point where the polar cell is formed might be first ventral, then posterior, then dorsal, and later anterior, and this would actually be the case in Asplanchna. In a special study of cleavage, with particular attention to direction, it is necessary that the orientation should give some *constant* basis for reference. It is therefore impossible for me to use Zelinka's orientation in my work. The animal pole of the egg *does* retain, however, a constant relation to the position of the blastomeres and to the axes of cleavage, so that I have adopted this relation as a basis for orientation.

4. CLEAVAGE.

Nomenclature.

For accurate comparative study of the direction and sequence of cleavage in the different regions of the egg, such a system of nomenclature is needed as will indicate directly the relationships, and especially the comparative age (measured in cell generations) of the blastomeres. The only system of nomenclature hitherto proposed which fulfils these demands is, I believe, that of Kofoid ('94). I shall therefore use his system in the following account.

The four blastomeres of the four-cell stage, and the cells derived from them, are designated respectively by the letters *A*, *B*, *C*, and *D*, beginning with the left anterior blastomere and passing around the egg to the right, i. e. in the same direction as the hands of a watch, — assuming the egg to be viewed from the animal pole. The letters thus represent the same blastomeres as in Wilson's work ('92) on Nereis, Heymons's ('93) on Umbrella, Lillie's ('95) on Unio, and Kofoid's ('95) on Limax.

After the first equatorial cleavage, at which the four original blastomeres are divided into smaller cells, the capital letters *A*, *B*, *C*, and *D* will be reserved to indicate respectively *all* the cells derived from the corresponding cell of the first four blastomeres; and such a collection will be called a *quadrant* of the egg.[1] The separate cells will be designated by the lower-case letters *a*, *b*, *c*, and *d*, according to the quadrant to which they belong. Each letter will be followed by two exponents. The first exponent indicates the generation to which the cell belongs, the unsegmented egg being considered the first generation. Thus, in the eight-cell stage (fourth generation) we shall have cells a^4, b^4, c^4, and d^4. Since, however, in this and in later generations, there are more than one cell of a given quadrant in a given generation, this first exponent must be followed by a second, serving to distinguish each cell from every other of the same quadrant. In "spiral" cleavage, this second exponent indicates the "quartet," or layer of cells, in the embryo to which the blastomere belongs, the *ventral* cell being number 1, the next dorsal number 2, and so to the most dorsal quartet. In equatorial cleavages the same relation may be preserved in other types of cleavage than the spiral. Thus, in the eight-cell stage (fourth generation), the ventral blastomeres are $a^{4.1}$, $b^{4.1}$, $c^{4.1}$, and $d^{4.1}$, while the corresponding dorsal cells are $a^{4.2}$, $b^{4.2}$, $c^{4.2}$, and $d^{4.2}$. But in meridional cleavages, where there is no trace of the so called spiral, this criterion fails, and the second exponent can be used only for distinguishing the cells, not for indicating their relative positions. What is required is a rational system of applying the exponent such that no two cells of the same quadrant in the same generation shall have the same exponent. Following the suggestion of Kofoid ('94), I have in meridional cleavages designated the *right* derivative with the *even* exponent in *even* generations, and with the odd exponent in odd generations, — the left derivative of course receiving the reverse designation. This method of application was designed to preserve any possible homologies of the products of meridional with those of spiral cleavage, since in normal spiral cleavage the right derivative lies above the left in even generations, and so receives the even exponent, while in odd generations the reverse is true. The results, however, have not shown any striking homologies with spiral cleavage, but the method of application has been retained, since no other seems to have any advantage over it.

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[1] Each quadrant from the four-cell stage onward receives a specific color in the plates, so that the quadrants are instantly distinguishable by their colors.

In meridional cleavages, the terms *right* and *left* will be used as defined by Kofoid ('94, p. 180): "A miniature observer is imagined as placed in the principal (vertical) axis of the egg, with his head at the animal pole, facing the part or parts under consideration, and the terms *right* and *left*, *upper* and *lower*, are used as determined by this observer."

A full account of this system of nomenclature is given by Kofoid ('94). In order to make clear the relation of the succeeding blastomeres and their designations by this system, I give here a scheme of the nomenclature through the sixth generation, modified from that given by Kofoid. Only the products of quadrant *A* are carried out beyond the third generation, since the method is the same for the other quadrants. Here $a^{6.1}$ represents the most ventral cell derived from the blastomere A, while $a^{6.8}$ represents the most dorsal one, the others occupying intermediate positions.

1 cell.	2 cells.	4 cells.	8 cells.	16 cells.	32 cells.
$\overline{ABCD}^1$	$\overline{AB}^2$	A^3	$a^{4.2}$	$a^{5.4}$	$a^{6.8}$
					$a^{6.7}$
				$a^{5.3}$	$a^{6.6}$
					$a^{6.5}$
			$a^{4.1}$	$a^{5.2}$	$a^{6.4}$
					$a^{6.3}$
				$a^{5.1}$	$a^{6.2}$
					$a^{6.1}$
		B^3			
	$\overline{CD}^2$	C^3			
		D^3			

First Cleavage.

After the formation of the polar cell the nucleus (Plate **1**, Fig. 2) returns to the position formerly occupied by the germinal vesicle in the longitudinal axis of the egg, lying nearer that end of the ovum in proximity to which the polar cell was formed (the micromere end). It takes such a position that a plane at right angles to the long axis of the egg and cutting the polar cell would also cut the nucleus. The distance from the centre of the nucleus to the nearer end of the egg is about two fifths of the length of the egg. Here two asters appear on opposite sides of the nucleus, the line joining them being oblique to the long axis of the egg. Though I have examined a large number of eggs at this stage, in no case have I been able to observe a stage in the process of forming the two asters of the first cleavage spindle (Fig. 3) from the single aster remaining after the formation of the polar cell (Fig. 2).

Between the two asters a spindle is formed. This lies at first somewhat oblique to the longitudinal axis of the egg, as shown in Figure 3, but before cleavage takes place the spindle swings into coincidence with the long axis (Fig. 4). The aster lying at the micromere end of the spindle is distinctly smaller than the opposite one (Fig. 3). The nucleus becomes divided into two small masses, which move toward opposite ends of the egg, but remain connected for a time by a distinct strand (Fig. 4). Meantime, before the first cleavage plane has appeared in the cytoplasm, the aster of the smaller blastomere has begun to divide, as shown in Figure 4. The two resulting asters separate at right angles to the axis of the first cleavage spindle. In the future larger cell the aster does not begin at once to divide. Both nuclei begin immediately to increase in size. The first cleavage plane passes through the point on the surface of the egg marked by the polar cell, transversely to the long axis of the ovum, and through about the middle of the strand connecting the two nuclei. The strand is slightly thickened at the point where the first cleavage plane is to meet it (Fig. 4), indicating perhaps the formation of the "Zwischenkörper." The cleavage plane is thus perpendicular to the axis of the spindle, and passes through its middle. I mention this fact on account of the difference between the first cleavage of Asplanchna and that of Callidina. In the latter rotifer, according to Zelinka ('91), the first cleavage plane is oblique to the spindle, and the spindle itself, even at the time of division, is oblique to the long axis of the egg. In another rotifer, Eosphora, the first cleavage plane is likewise oblique to the long axis of the egg (Tessin, '86), while in Melicerta ringens (Zelinka, '91) and Asplanchna Sieboldii (Lameere, '90) the first cleavage plane is transverse to the long axis, as in Asplanchna Herrickii.

During and after the passage of the first cleavage plane through the cytoplasm, the egg retains its ellipsoidal form, and the resulting cells do not separate and become rounded, as occurs in the eggs of so many animals, but remain closely pressed together. In a large series of cases showing the first cleavage in various stages, the only indication of any change in the form of the egg or its blastomeres is a slight depression of the surface where the cleavage plane cuts the periphery of the egg, forming a shallow furrow. Here the edges of the two blastomeres are slightly rounded off, as shown in Figure 6, instead of fitting squarely against each other. The retention of its general form by the egg is characteristic of all cleavage stages. This surface of contact of the two blastomeres is curved, the smaller cell, $\overline{AB}^2$, projecting slightly into the larger.

So far as the direction of the division is concerned, the first cleavage of Asplanchna evidently fits easily either the surface tension theory of Berthold, or Hertwig's theory of the spindle in the long axis of the protoplasmic mass. Comparison with the first division of Callidina russeola as described by Zelinka ('91) develops an interesting fact. In

Callidina the first cleavage spindle is *oblique* to the long axis of the egg, therefore not in agreement with Hertwig's law; but immediately after division is finished, a movement of the egg contents takes place in such a way that the two cells occupy the same relative position as in Asplanchna, — such a position, therefore, as is demanded by Berthold's theory of least surfaces. It thus appears that in Callidina the direction of division itself is determined neither by the principle of Berthold nor that of Hertwig, but that the later arrangement of the cells might be held to be due to the action of Berthold's principle. It is somewhat curious that the exact arrangement produced in Callidina by shifting should in Asplanchna result at once from the position of the spindle at the time of cleavage.

No cause can be assigned, from the visible structure of the egg, for the inequality of the cleavage. The yolk granules are distributed uniformly throughout the egg, seeming no more abundant in the large than in the small cell.

Second Cleavage.

As a result of the first cleavage, the egg is now composed of two unequal blastomeres, an anterior, $\overline{AB}^2$, and a posterior, $\overline{CD}^2$ (Figs. 5 and 6).

In the smaller blastomere, as previously stated, the aster has already divided and the two parts are separating at the time when the first cleavage plane passes through the cytoplasm (Fig. 4). The line along which they move apart is perpendicular to the axis of the first cleavage spindle, and also at right angles to a line connecting the polar cell with the centre of the egg. The forming spindle is thus parallel to the lateral axis of the embryo and consequently perpendicular to its dorsoventral axis. The two asters take up their positions on opposite sides of the nucleus, and the axis of the resulting spindle has a direction parallel to the line joining the asters at their first separation (Figs. 5 and 6). Meanwhile the nucleus has steadily increased in size, up to the time when it participates in the formation of the spindle.

In the larger cell, $\overline{CD}^2$, the order of procedure is different. The nucleus begins to enlarge, as in the smaller blastomere, but the aster does not at once divide. The nucleus and aster together begin to migrate to the right. At the same time the aster comes to lie farther to the right than the nucleus, either because the two rotate on a common axis, or because the aster, moving faster, creeps around the nucleus toward the right side of it. Thus, whatever the method, a condition is reached in which the large nucleus lies in the right anterior angle of the cell $\overline{CD}^2$, with the undivided aster at its right and slightly behind it (Fig. 5).

By this time the two asters in the cell $\overline{AB}^2$ have completely separated and lie upon opposite sides of the enlarged nucleus. Thus the preparatory stages for karyokinesis are much more advanced in the smaller cell, and it would be anticipated that this cell would cleave first.

The single aster in $\overline{CD}^2$ now begins to divide. The process seems to be accomplished very quickly, since in a series of nineteen specimens of the two-cell stage (each, of course, taken from a different individual) only one case was found exhibiting a transitional stage between that shown in Figure 5 and that shown in Figure 6. In this specimen the single aster had elongated slightly in the direction of the future spindle. When formed, the spindle takes an oblique position in the cell, extending from right anterior to left posterior. The aster at the left posterior end of the spindle is much the larger, in correlation apparently with the larger mass of cytoplasm surrounding it. The nucleus of $\overline{CD}^2$ has now overtaken in its metamorphosis that of $\overline{AB}^2$; the spindles are found in exactly corresponding stages, the chromatin being in both arranged in an equatorial plate (Fig. 6).

Not only are the two spindles not parallel, as shown in Figure 6, but they do not lie in the same plane. If the two spindles are viewed exactly from the anterior or from the posterior end of the egg, the left aster in $\overline{AB}^2$ and the right aster in $\overline{CD}^2$ are seen to lie more dorsally than their mates. Viewed in this direction, the spindles cross each other at an angle of about twenty-five degrees.

As a result of the dissimilarity in the direction of the two spindles, the two next cleavage planes, perpendicular to them, will not meet the first cleavage plane in a common line. The position and direction of the spindle in $\overline{CD}^2$ are such that the cleavage plane cutting $\overline{CD}^2$ would probably meet the first cleavage plane to the right of the line where the plane dividing $\overline{AB}^2$ would meet it. Since the right aster of $\overline{CD}^2$ is farther dorsal than the left, the plane of cleavage of $\overline{CD}^2$ would be inclined to the sagittal plane, — on the dorsal side toward the left, on the ventral side toward the right.

The cleavage of the two cells now follows at almost or precisely the same time, the karyokinetic processes being found from this time on in the same stage. In a series of thirty-one eggs from different individuals, each containing more than one and less than five cells, none contained exactly three cells.

An examination of the four-cell stage after the completion of division

(Plate **2**, Fig. 8) shows that the cleavage planes have taken the positions foreshadowed by the arrangement of the spindles. The plane separating C^3 from D^3 lies to the right of the plane separating A^3 from B^3; the corresponding furrows on the surface are nearer together on the dorsal than on the ventral side. The blastomeres resulting from the division of $\overline{AB}^2$ are equal, whereas $\overline{CD}^2$ divides very unequally. The right derivative (C^3) is much smaller than the left (D^3), and is of approximately the same size as A^3 and B^3. The blastomeres B^3 and D^3 are in contact along the whole distance from the dorsal to the ventral surface of the egg, while A^3 and C^3 do not touch each other at all. The polar cell lies either at the junction of B^3, C^3, and D^3, as shown in Figure 8, or sometimes at the junction of A^3, B^3, and D^3. The egg is now markedly unsymmetrical.

It is evident from the above description that this cleavage may be considered as belonging to the so called spiral type. Since the left end of the spindle is in each cell the higher, the cleavage is a left spiral, like the corresponding cleavage in Discocelis, Nereis, Limax, and indeed all forms with spiral cleavage except in the reversed cleavage of certain mollusks. This fact is striking, since the succeeding cleavages in Asplanchna *do not belong to the spiral type.*

The relation between the axes of the embryo in later stages and the first two cleavage planes is as follows. The first furrow separates an anterior from a larger posterior portion, but the plane of separation of the parts bears no simple relation to the axes of the later embryo. (Compare Figure 8 with Figure 75, Plate **9**, in which the parts derived from the first four cells are colored in the same manner as their parent cells in Figure 8, and note the great shifting.) The later sagittal plane of the embryo is coincident with a plane passing through the animal pole and the longest axis of the egg; that is, through the plane separating A^3 from B^3 (Fig. 8), and dividing the larger blastomere D^3 into two unequal parts. The second cleavage plane therefore divides the right side from the left in the anterior part of the egg; but in the posterior part it lies entirely in the right side. It is not until the seventh cleavage that the division into symmetrical right and left halves takes place on the posterior side (Plate **7**, Fig. 58); indeed, certain cells containing material for both sides of the egg remain undivided till even a later stage.

Third Cleavage.

Immediately after the second cleavage, the aster in each of the four cells produced begins to extend dorso-ventrally, at right angles to the axis of the preceding spindle. An optical section of the egg along its chief axis, showing the asters in the cells B^3 and D^3, is given in Plate **2**, Fig. 10. Spindles are formed in all four cells nearly or quite in the position indicated by the direction of separation of the asters.

The tendency of the karyokinetic processes in the posterior half of the egg to gain upon those in the anterior half, shown during the last division, is continued and accelerated. Spindles appear in C^3 and D^3, while the nuclei in A^3 and B^3 are still spherical and have distinct membranes. Figure 9 gives a view of this stage from the right side; the large spherical nucleus of B^3 is represented by a broken outline. The spindle in C^3 has a dorso-ventral direction, and its middle coincides with the middle of the length of the cell; the two asters are of equal size. In D^3 the spindle is nearer the dorsal side of the egg, and is inclined, passing from dorsal and anterior to ventral and posterior. The ventral aster is the larger.

Cleavage takes place first in the larger cell D^3, separating a large ventral blastomere, $d^{4.1}$, from a smaller dorsal one, $d^{4.2}$. At the same time the two cells (considered as a whole) elongate dorso-ventrally. In so doing, the ventral blastomere, $d^{4.1}$, remains nearly stationary, while $d^{4.2}$ moves in the direction of the animal pole of the egg. (Compare Figure 12, a sagittal section of a five-cell stage, with Figure 10, the corresponding section of a four-cell stage, observing the position of the cells in relation to the general form of the egg.) As a result of this, the dorsal end of the cell B^3, and, to a less degree, the ends of A^3 and C^3, are displaced in the same direction; that is, the whole animal pole moves toward the micromere end of the egg. At the same time the cells A^3, B^3, and C^3 are slightly compressed dorsoventrally. This is the beginning of that peculiar rotation of the blastomeres in the eggs of Rotifera, described by Zelinka ('91) and others, which eventually results in the process of gastrulation.

C^3 divides next, the cleavage being equal; the products are $c^{4.1}$ and $c^{4.2}$.

Before the cleavage is finished in D^3 and C^3, spindles have been formed in B^3 and A^3, division taking place in them in the order named. The cleavage is equal, as in C^3.

The order of cleavage, then, for the four cells, is as follows: *D*, *C*, *B*, *A*. This rhythm reappears in later cleavages.

The third cleavage is therefore equatorial, dividing the egg into two layers of four cells each. The ventral cells are $a^{4.1} - d^{4.1}$, the dorsal cells $a^{4.2} - d^{4.2}$. The egg is still slightly unsymmetrical.

Views of the eight-cell stage are shown in Figures 15 to 18.

From a cyto-mechanical standpoint, the third cleavage may be characterized as follows. The first division of the asters is along a line at right angles to the axis of the previous spindles, and indicates the position of the spindles for the next cleavage. These lie in the long axes of the cells, and the cell walls are formed in the position demanded by the principle of least surfaces.

Fourth Cleavage.

Immediately after the division of D^3 (Plate **2**, Fig. 12), the asters in $d^{4.1}$ and $d^{4.2}$ begin to extend laterally, at right angles to the axis of the preceding spindles, and each becomes divided into the two asters for the following spindle. In $d^{4.1}$, Figure 11, the two asters for the succeeding cleavage are still connected by a striate band. Figure 11 shows a ventral view of the same egg as Figure 12, the five-cell stage. The corresponding dorsal view of a slightly later stage is shown in Figure 14. The asters in $d^{4.2}$ are moving apart in the same manner as in $d^{4.1}$, save that the line of separation is slightly oblique, the left aster being higher.

In the same way the asters in the cells $a^{4.1}-c^{4.1}$ and $a^{4.2}-c^{4.2}$ become constricted, and divide at right angles to the axis of the preceding spindles. The dividing asters in $c^{4.2}$ are shown in Figures 14 (Plate **2**) and 17 (Plate **3**), and those of $c^{4.1}$ in the latter figure. Views of the other four cells would show similar conditions.

From the manner in which the asters separate in all of the eight cells, one would be led to expect that the next cleavage would be meridional, at right angles to the third cleavage. This expectation is strengthened by the fact that the *lateral* dimensions of the cells in which the asters lie are considerably greater (in the quadrants *A*, *B*, and *C*, at least) than the opposite measurements (Fig. 17).

But in a slightly later stage it is observed that the line joining the asters in $d^{4.1}$ has become oblique, like that joining those of $d^{4.2}$ (as mentioned above). This oblique position of the asters in $d^{4.1}$ is shown in the ventral view (Fig. 15). The left aster (right side of the figure) has become ventral, the right one dorsal. The sagittal section (Plate **1**, Fig. 7) of a slightly later stage shows the completion of the rotation thus begun; the line connecting the two asters and passing through the nucleus is now approximately dorso-ventral in direction.

At the same time a similar rotation has taken place in the cell $d^{4.2}$, but the position taken by the two asters is not the same as in $d^{4.1}$. One of the two asters has become central, the other peripheral, as if the cell were about to divide into a deep and a superficial portion. This condition also is shown in the sagittal section, Figure 7. The difference in the position of the asters in $d^{4.1}$ and $d^{4.2}$ is apparently due to simple mechanical conditions, — the form of the cell $d^{4.2}$ compelling the asters to take the position which they have.

At the same time a slight differentiation in the cytoplasm of the cell $d^{4.1}$ becomes visible. As previously stated, the fine yolk granules are at first distributed uniformly throughout the egg. In this eight-cell stage, a slight concentration of the yolk granules in the ventral part of the cell $d^{4.1}$ may be noticed by careful observation. The condition at this time is shown in Figure 7; in the ventral part of $d^{4.1}$ the yolk granules are a little larger and more numerous. As will be shown, this concentration of yolk becomes later much more marked, and its history is peculiar.

A spindle is now formed in $d^{4.1}$ in the position indicated by the asters of that cell in Figure 7, — that is, with a dorso-ventral axis, — thus prefiguring another *equatorial* cleavage. The spindle is shown in Plate **2**, Figure 16.

Immediately thereafter the spindle is formed in $d^{4.2}$, and it appears that the position of the asters shown in Figure 7 (Plate **1**) is *not definitive*. The asters shift, so that the spindle in $d^{4.2}$ is dorso-ventral, like that in $d^{4.1}$, as is shown in Figure 16. Which of the two asters seen in Figure 7 becomes dorsal and which ventral, I have been unable to determine. During the formation of the spindle in $d^{4.2}$ the cell extends a little in the direction of the spindle, as is shown by a comparison of Figure 7 with Figure 16.

Meanwhile, changes have been occurring in the quadrants *A*, *B*, and *C*. As the processes are the same in all three, the quadrant *C* will be selected as a type.

At first, as described above, the asters separate tangentially, at right angles to the axis of the previous spindle (Plate **3**, Fig. 17). This position is retained for some time, but in a later stage the line connecting the asters in $c^{4.2}$ has become oblique, as shown in Figure 18, which exhibits a side view of the egg of which Figure 7 is a section. The asters in $c^{4.1}$ still retain their original position.

Now follows the cleavage of the cell $d^{4.1}$. This is accompanied by an increase of the dorso-ventral extent of the two products, as compared with that of the original cell. The division is unequal; the ventral cell $d^{5.1}$ is much the larger, and retains the whole of the territory containing the larger yolk granules shown in Figure 7. The larger derivative retains its position at the macromere end of the egg (Fig. 19). The smaller cell $d^{5.2}$ is therefore pushed dorsad, and this, together with the extension of $d^{4.2}$ at the time of the formation of its spindle, displaces the animal pole, marked by the polar cell, still farther toward the micromere end of the egg. As a further result, the cells of the quadrants *A*, *B*, and *C* are still more compressed dorso-ventrally, so that, especially in $a^{4.1}-c^{4.1}$, the lateral extent is much greater than the dorso-ventral (Fig. 19).

Nevertheless, as Figure 19 shows, the rotation of the future spindle axis still continues. The line joining the asters becomes dorso-ventral first in the dorsal cells $a^{4.2}-c^{4.2}$, while in $a^{4.1}-c^{4.1}$ the asters are still oblique, as shown in $c^{4.1}$, Figure 19. In quadrant *B* of this same figure, the axis has become dorso-ventral in both cells.

Now occurs the cleavage of $d^{4.2}$, with still further elongation, shifting of the animal pole toward the micromere end of the egg, and resulting greater compression of the cells of the quadrants *A*, *B*, and *C* (Figs. 20–24). Without regard to this, the asters in the cells of these quadrants continue their movements until the future spindle axes are in every case dorso-ventral. Spindles are now formed in all of the six cells, the spindle being in every case in the shortest axis of the cell (Figs. 20–24).

The conditions at this stage are so significant from a cyto-mechanical standpoint, that I have thought it best to analyze and illustrate with especial fulness a typical egg at this stage. Figures 20–25 are views of a single egg. Figure 20 shows the right side (quadrant *C*), Figure 22 the left side (quadrant *A*), Figure 21 an anterior view (quadrant *B*), and Figure 25 a posterior view (quadrant *D*). In Figures 23 and 24 are given respectively sagittal and frontal optical sections, for comparison with the surface views.

In order to exclude possibility of error, the egg from which the above figures were taken was moved about so that the six cells belonging to the quadrants *A*, *B*, and *C* occupied successively the middle of the upper surface of the egg; careful camera figures of each cell were made in this position. Then optical sections were taken in the same way, both along the axis in which the spindles lie, and at right angles to these. Accurate measurements of the dimensions of the cells could thus be made; the results are as follows.

Cell.	Dorso-ventral Measurement.	Lateral Measurement.	Ratio of Dorso-ventral to Lateral Measurement.
$a^{4.1}$	25μ (20μ[1])	52μ	1 to 2 (about) (2 to 5[1])
$a^{4.2}$	35μ	50μ	7 " 10
$b^{4.1}$	24μ	42μ	4 " 7
$b^{4.2}$	35μ	42μ	5 " 6
$c^{4.1}$	30μ	49μ	3 " 5 (about)
$c^{4.2}$	33μ	52μ	2 " 3 (about)

After the spindles become completely formed, the cells begin to elongate in the direction of the spindles. A slightly later stage than that just described is shown in Plate **4**, Figure 26. Comparing this with Plate **3**, Figure 22, it is evident that the cell $a^{4.1}$ has stretched in the direction of the spindle to such an extent that the difference between the two axes of the cells is much diminished. Nevertheless, in both this cell and $a^{4.2}$ the axes in which the spindles lie are distinctly the shorter. This is still true at the time of the division of the cells. Figure 27 (Plate **4**) shows the right side of the egg last considered; in the quadrant *C* the processes are much more advanced than in *A*. The nuclei have separated and the cytoplasm is dividing, yet exact measurements both of surface views and optical sections show that the greater diameter is still at right angles to the line joining the two nuclei. A frontal section, showing the greatest dorso-ventral extent of the cells of the quadrants *A* and *C* of this egg, is given in Figure 28.

The two figures last mentioned show another fact of importance. The divisions do not separate the blastomeres into cells of equal size in the quadrants *A*, *B*, and *C*. The completed cleavage is shown in Plate **4**, Fig. 30 (anterior view). This, with the figures just cited, shows that the cells $a^{4.2}-c^{4.2}$ divide very unequally, the dorsal derivatives, $a^{5.4}-c^{5.4}$, being very much larger than the ventral ones, $a^{5.3}-c^{5.3}$. The inequality is less in the division of the ventral cells $a^{4.1}-c^{4.1}$. Although the ventral derivatives, $a^{5.1}-c^{5.1}$, occupy a larger area on the surface of the egg, there is little difference in actual volume, and such as occurs is in favor of the more dorsal cells $a^{5.2}-c^{5.2}$.

The order of division is the same as in the last cleavage; first, the quadrant *D*, then in order *C*, *B*, *A*. In quadrant *D* the larger cell $d^{4.2}$ divides first; in the other quadrants the cells are of equal size and divide at the same time.

The important facts in this fourth cleavage, from a cyto-mechanical standpoint, may be summarized as follows.

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[1] In every case the first measurement was taken through the two asters; in the case of $a^{4.1}$ the real dorso-ventral extent of the cell, into which the spindle later moves, is but 20μ, — so that the ratio is as two to five.

The asters in all of the eight cells after the third cleavage separate tangentially, at right angles to the direction of the preceding spindles. Having taken up positions on opposite sides of the nucleus, in every case the complex of nucleus and asters *rotates* in such a way as to bring the axis of the forming spindle into the same direction as that occupied by the spindle for the preceding cleavage. In six of the cells, this rotation is from a previous position in the *longer* axis of the cell to a later position in the *shorter* axis. In these six cells spindles are completed in the shortest axes of the cells, and division ensues in such a way that the newly formed septa are surfaces of *greatest area,* and the cells separate in the direction of *greatest pressure.*

The cleavage in $a^{4.2} - c^{4.2}$ and in $d^{4.1}$ is markedly unequal.

In two cells of equal ages but unequal size ($d^{4.1}$ and $d^{4.2}$) the larger divides first.

The exact changes in form during the divisions of the cells is a point worthy of careful attention. As the transformation of the nucleus giving rise to the spindle takes place, the cell elongates slightly in the direction of the spindle. (Compare $c^{4.1}$, Figure 24, with the earlier stage of the similar cell $a^{4.1}$ in the same figure.) As the spindle narrows and lengthens and the chromosomes begin to separate, the cell continues to elongate (Fig. 26, $a^{4.1}$, compared with Fig. 20, $c^{4.1}$, and Fig. 22, $a^{4.1}$). As the two new nuclei are formed and move apart, and the cytoplasm becomes constricted, there is a still further extension of the cells in the direction of the spindle. (Compare $c^{4.1}$ and $c^{4.2}$ with $a^{4.1}$ and $a^{4.2}$, in Plate **4**, Fig. 28.)

As Heidenhain ('94^a, p. 154) has recently urged, this elongation of the cell in the direction of the spindle is a point of great importance for a proper understanding of the conditions affecting the direction of cell division. In many later divisions in Asplanchna the spindle is first formed, as will be shown, in the short axis of the cell, and then this axis by stretching becomes the longer. It is possible that to this phenomenon is due the apparent general agreement of normal cleavage with the law of Hertwig, and that careful observation will in many cases, as in Asplanchna, show the so called law to be of little significance.

A full discussion of the bearing of the facts above described is reserved until later cleavages have been examined.

The foregoing description is based on a study of forty-two specimens from different individuals, showing the various phases of the fourth cleavage; that is, each containing more than seven and less than sixteen cells.

This cleavage, as described above, differs in some respects from that of Callidina as described by Zelinka ('91). A discussion of the differences will be found in Part Second.

The cleavage of Asplanchna priodonta takes place to this stage in exactly the same manner as that of Asplanchna Herrickii. Figure 29 (Plate **4**) shows the egg of Asplanchna priodonta in the 10-cell stage.

Fifth Cleavage.

As a basis for an account of the following cleavage, it will be well to summarize the divisions which have already taken place, and to take a careful survey of the structure of the egg at the end of the fourth cleavage.

The first and second cleavages pass through both animal and vegetative poles and are therefore meridional. The third cleavage is at right angles to the dorso-ventral axis and is therefore equatorial. The fourth cleavage is parallel to the third, thus likewise equatorial.

As a result of these cleavages, the egg now consists of sixteen cells, arranged in four dorso-ventral rows or *quadrants*, each quadrant consisting of four cells derived from one of the four blastomeres of the four-cell stage (Plates **4** and **5**, Figs. 30–36). Passing from the ventral side dorsad, we may also distinguish four *layers* of cells, each layer containing one cell of each of the four quadrants. The layers may for convenience be numbered; I will call the ventral the *first* layer, the others following in order to the *fourth*, which is at the animal pole. As a result of the shifting during cleavage, the animal pole has now come to be situated almost exactly at the micromere end of the egg; the opposite end is occupied by the large cell $d^{5.1}$ (Figs. 30 and 33, anterior and posterior views respectively). The dorso-ventral axis therefore now coincides with the long axis of the egg.

Of the four quadrants, three, *A*, *B*, and *C*, are alike in the size and arrangement of the cells of which they are composed. (See Fig. 30, anterior view.) The four cells composing any given one of these quadrants differ in size. The cell of the fourth layer (next to the animal pole) is much the largest, while that of the third layer is much the smallest. The cells of the first and second layers are nearly equal in size; that of the first layer covers more of the surface of the egg (Fig. 30), but that of the second layer is deepest (Fig. 32). The cells of the first, second, and third layers are much compressed dorso-ventrally, so that the lateral dimensions of the cells are at least twice as great as the dorso-ventral dimensions. In the third layer especially, the cells are deformed by the pressure to such an extent that the surface area exposed resembles the section of a biconvex lens. In the dorsal layer the compression is less; the cells are triangular in surface view, and the dorso-ventral extent is greatest.

All the cells of quadrant *D* are much larger than the corresponding cells of the other quadrants (Figs. 31 and 33). The ventral blastomere, $d^{5.1}$, is much the largest cell of the egg, and occupies the entire ventral end at this period. Its position as shown by the section (Fig. 32) is worthy of careful attention. Its dorsal or inner surface, like the outer, is convex; anteriorly the cell is partly covered by the ventral cells of the other quadrants, while the ventral end of cell $d^{5.2}$ extends a slight distance ventrad of the middle dorsal portion of $d^{5.1}$.

The cell $d^{5.1}$ is distinguished from all the others by a further peculiarity. I have shown above (page 25 and Fig. 7) that in the eight-cell stage there is a slight concentration of yolk material in the ventral region of the cell $d^{4.1}$, where the yolk granules are a little larger and more numerous. At the division of $d^{4.1}$, this cloud of granules, as a natural result of its position, remains in the cell $d^{5.1}$ (Plates **2** and **3**, Figs. 16 and 19). At the same time it becomes more distinctly differentiated. The granules composing the cloud increase in size and range themselves about the periphery of the egg, next to its free surface (Figs. 23, 24, Plate **3**, and Fig. 28, Plate **4**). A narrow strip of the posterior margin of the free surface of the cell is without the granules (Figs. 20, 22, and 23). At a time when the fourth cleavage is entirely completed, the granules have withdrawn still farther from the posterior margin of the cell, and show a tendency to concentrate at the free surface of the cell over its anterior half (Plate **4**, Fig. 32). In the other cells, and in the remaining portions of $d^{5.1}$, the original finely granular cytoplasm is retained, so that I have not thought it necessary to represent in the figures the yolk conditions in any region except where the cloud of granules is present.

The cell of the second layer, $d^{5.2}$, is next in size, then the dorsal cell, $d^{5.4}$, while the cell of the third layer is the smallest in quadrant *D*. The cells $d^{5.2}$ and $d^{5.3}$ are very greatly compressed dorso-ventrally and elongated laterally, so as to form irregular flat plates, extending from the posterior surface of the egg two thirds of the distance to the anterior surface. (Compare Figure 31, left posterior surface, with Figure 32, section.) The dorsal cell $d^{5.4}$ is likewise compressed dorso-ventrally, so as to appear in a sagittal section (Fig. 32) as a low triangle.

As a whole, the form and arrangement of cells are far from what is demanded by the principle of least surfaces. Flat plates, such as we see in $d^{5.2}$ and $d^{5.3}$, and in all the cells of the third layer, retain their form in virtue of some force working strongly *against* surface tension.

After the fourth cleavage, the asters in all the cells at first separate at right angles to the axes of the preceding spindles, as happened after the third cleavage. The later changes are essentially the same in all the quadrants, so far as the asters are concerned, so that quadrant *D* may be described as a type.

Figure 31 (Plate **4**) shows the conditions in the four cells of quadrant *D*, after the asters have divided. The two asters of each cell lie upon opposite sides of their nuclei in such a position that, if no change occurs, the ensuing division will be meridional.

In the cells of the first three layers the asters retain their original positions. But in $d^{5.4}$ a rotation takes place, such as occurred in all the cells in preparation for the preceding (fourth) cleavage, so that the axis of the spindle in $d^{5.4}$ is at right angles to the axes of the spindles in the other three cells of the quadrant. This condition is shown in Figure 33, and the completion of the division is shown in Plate **5**, Fig. 37.

The same processes take place in the other quadrants, so that all the cells of the first three layers have spindles extending laterally, while in the fourth or dorsal layer the spindles are directed dorso-ventrally (Fig. 40).

We must now consider the cleavage in the several cells more in detail.

As in previous cleavages, division takes place first in the cells of quadrant *D*. The nucleus of the large ventral cell, $d^{5.1}$, is earliest to enter upon the karyokinetic process, followed immediately by $d^{5.2}$, and a little later by $d^{5.3}$ and $d^{5.4}$. Figure 33 (Plate **4**) gives a view of the posterior surface of the egg at this stage, showing the spindles in all the cells. As this figure shows, the spindles in the three cells $d^{5.1}$, $d^{5.2}$, and $d^{5.3}$ do not lie in the middle of the cells, but nearer the right ends. (See definition of right and left, page 17.) In the dorsal cell, $d^{5.4}$, the spindle is at right angles to those in the other cells. The plane of cleavage indicated in the three ventral cells is meridional; in the dorsal cell it is equatorial, like that of the two preceding cleavages. The division of each of the four cells must be considered separately.

To understand the cleavage of the large ventral cell, $d^{5.1}$, it is necessary to observe accurately its position and relation to the other cells. A longitudinal section of about the same stage as that shown in Figure 33 is given in Figure 34. Comparing this with the earlier corresponding section, Figure 32, it is seen that with the formation of the spindles in $d^{5.2}$ and $d^{5.3}$ these cells have yielded to the well known tendency to take a more rounded form at the time of karyokinesis; the inner parts of the cells have been withdrawn toward the surface and used in increasing the dorso-ventral dimensions of the cells. The animal pole has been thereby pushed still farther in the direction in which it has been steadily migrating, so that it is actually past the micromere end of the egg. The cells of the quadrants *A*, *B*, and *C*, being in a "resting" condition, give way to the compression, and become much deeper and flatter than before. The cell $d^{5.1}$ retains its position at the macromere end of the egg, but lying in a concavity, partly surrounded by the other cells. The spindle lies in the deeper (more dorsal) parts of the cell, with its right end (Fig. 33) deepest, and close to the wall of the cell. A view from the ventral end of the egg (Plate **5**, Fig. 35) shows that this "right" end is really anterior, and that the spindle lies in an antero-posterior plane, coincident with the plane separating the quadrants *A* and *B*. The anterior (inner) end of the spindle lies close against the boundary between $a^{5.1}$ and $b^{5.1}$.

The division which now ensues is of an extraordinary character. The anterior end of the spindle is pressed against the periphery of the cell at the place above mentioned, and a minute vesicle is given off, which lies embedded between the cells $a^{5.1}$ and $b^{5.1}$. This, after the division is finished, is shown in Figure 38 (Plate **5**), the vesicle being labelled $d^{6.2}$.

During division, the granular cloud which was described as occupying the anterior half of the periphery of the cell moves still farther toward the anterior margin, and shows a tendency to concentrate into a more definite group; the individual granules become larger also (Fig. 38).

In $d^{5.2}$ and $d^{5.3}$ the spindles are parallel to the spindle in $d^{5.1}$, the right ends being nearer the boundary of the cells, and deeper within the egg. The latter fact is shown in the transverse section, Figure 36, passing through the cells of the third layer. The divisions are unequal, as foreshadowed by the position of the spindles, but the inequality is much less than in the case of $d^{5.1}$. The completed division is shown in Figure 37. The cleavage takes place first in $d^{5.2}$.

At about the same time as the division of $d^{5.3}$ occurs that of the dorsal cell, $d^{5.4}$. Here the spindle is in the short axis of the cell; the cleavage is equatorial and unequal, the dorsal cell being much the smaller (Fig. 37).

During the occurrence of the cleavage of these cells other changes, which are of the greatest importance, have been taking place, partly as a consequence of these cleavages. As the constriction of the cytoplasm in $d^{5.2}$ and $d^{5.3}$ occurs, these cells show in a most pronounced way the tendency to become of a rounded form. The inner portions of the cell are withdrawn still more from the centre of the egg, until the antero-posterior measurements are no greater than their dorso-ventral dimensions. This is shown in the section, Figure 38. In this egg, a surface view of which is given in Figure 37, the cleavage of $d^{5.2}$ is finished, and the products, $d^{6.3}$ and $d^{6.4}$, have already passed into the "resting stage," so that they take whatever form is impressed upon them by the surroundings. But $d^{5.3}$ is just dividing into $d^{6.5}$ and $d^{6.6}$, and the form shown in section by $d^{6.5}$ in Figure 38, as compared with the form of $d^{5.3}$ in Figure 32, shows the change which I have been describing.

At the same time the cells of the other quadrants, *A*, *B*, and *C*, are entering upon the stages preparatory to karyokinetic division. As a first step they also retract their deeper parts and bring their protoplasm into a more compact mass, as shown by a comparison of quadrant *B* in Figure 34 (Plate **4**) with the similar quadrant, *A*, in Figure 38 (Plate **5**).

As a consequence of this withdrawal of material from the inner parts of the egg, the large ventral cell $d^{6.1}$, which has now passed into the resting stage, moves inward to occupy the space which would otherwise be vacant, — being forced to do so, of course, by the greater dorso-ventral extension of all the other cells. The result is shown in Figure 38. This partial enclosure of $d^{6.1}$ by the other cells is of course a stage in the process of gastrulation.

Before the cleavage of all the cells of quadrant *D* is finished, the karyokinetic processes have begun in the other three quadrants. (See Plate **5**, Figs. 39–42.) The first cells to show the characteristic nuclear phenomena are those of the fourth or dorsal layer, $a^{5.4} - c^{5.4}$. As previously stated, the asters at first take up such a position in these cells as would lead, if unchanged, to a meridional cleavage. But in these three cells, as in $d^{5.4}$, there is a revolution of the asters and nuclei, resulting in a dorso-ventral position of the spindles. At any given period the three cells are not in exactly the same phase of division, though very nearly so; the order, beginning with the most advanced, is $c^{5.4}$, $b^{5.4}$, $a^{5.4}$. The sequence is thus the same as in previous cleavages. The division is equatorial and the dorsal product is the smaller, as in the cleavage of the corresponding cell ($d^{5.4}$) of the left posterior quadrant. In the three cells of quadrants *A*, *B*, and *C* the spindles are not in the short axes, as in $d^{5.4}$, since these cells are not nearly so broad as that one, while the dorso-ventral dimension is about the same or greater (Plate **4**, Figs. 30 and 32). Figure 41 (Plate **5**) shows the process of cleavage in these cells, while Figure 45 (Plate **6**) shows the cleavage concluded.

The division of the second layer follows upon that of the fourth, and again in the order $c^{5.2}$, $b^{5.2}$, $a^{5.2}$. The division is here meridional, as it is in the corresponding cells of the quadrant *D*, and equal, as it is *not* in the corresponding cell ot the quadrant *D*. (See Figs. 39, 40, 43, and 44.)

Next follow the divisions of the cells of the first layer, in the same order as in the previous cleavages, and, slightly later, the divisions in the third layer, also in the same sequence. The cleavages here also are meridional and equal.

The nuclear conditions leading to these cleavages are shown in Figures 39, 40, 42 (Plate **5**), all from the same egg. A somewhat later stage is shown in Figure 43, which exhibits the conditions in the quadrants *A*, *B*, and *C* at a time when the divisions just described are completed in most of the cells. All the divisions are nearly finished except in the cells of the third layer ($a^{5.3}-c^{5.3}$), which still contain spindles. This view shows also another peculiar fact. During the cleavages the cells about the dorsal pole of the egg have shifted, and the cells $a^{6.7}$ and $c^{6.7}$ have pushed ventrad to such an extent that on the right side the cells $c^{5.3}$ and $b^{5.3}$ have become completely separated, a part of the cell $c^{6.7}$ lying between them. This condition is only transitory, however; the cell $c^{6.7}$ is very soon pushed dorsad again, and the cells of the third layer again form a continuous row. (Compare Figure 47, Plate **6**.) Figure 46 shows the cells of the quadrant *D* at the close of this division, while Figure 45 is a view of the animal pole at the same stage.

The features of the fifth cleavage may be summarized as follows. In the twelve cells of the three ventral layers, the asters separate after the fourth cleavage at right angles to the position of the preceding spindles and retain the position first taken ; the cleavage is therefore meridional. In all these cells the spindles are in the long axes of the cells. In the dorsal layer the asters at first assume the same position as in the other cells, but later a rotation takes place, and the spindles when formed have a dorso-ventral direction ; the resulting division is equatorial. The spindle is in the longer axis of the cells $a^{5.4}$, $b^{5.4}$, and $c^{5.4}$, in the shorter axis in $d^{5.4}$.

The division is unequal in the four dorsal cells of all the quadrants, and in all the cells of the quadrant *D*. In the other cells it is equal.

The sequence of cleavage is the same as in previous divisions, but with some modifications. In any given layer of cells, the order is *D*, *C*, *B*, *A*; a repetition of the sequence established at the third cleavage. In any given quadrant, the order of cleavage varies with the relative size of the cells. In the quadrant *D* the order is (beginning with the ventral cell) 1, 2, 4, 3, and this is also the order of relative size of the cells, beginning with the largest. In the other quadrants the order of cleavage is 4, 2, 1, 3, and this again is the order of comparative size beginning with the largest, except that 2 and 1 are so nearly of a size that it is difficult to say from observation that either is the larger.

The fifth cleavage is accompanied, as a result of the changes in form of the cells during karyokinesis, by a partial enclosure of the ventral cell of quadrant *D* ($d^{6.1}$) by the other cells.

The above account of the fifth cleavage is based upon an examination of twenty-five eggs, taken from different individuals and showing different phases of the division; i. e. each egg contained more than fifteen and less than thirty-two cells.

Sixth Cleavage.

The first division belonging to the sixth cleavage, that of $d^{6.1}$, takes place coincidently with the last division of the fifth cleavage, that of $a^{5.3}$. There is thus no resting period between the two cleavages. Nevertheless, there is a sufficiently well characterized stage of thirty-one or thirty-two cells, just as the cleavage of $d^{6.1}$ occurs, and it will be well to describe the egg in this condition as a basis for an account of the sixth cleavage.

Figure 43 (Plate **5**) shows the anterior surface just before this stage is attained; Figure 47 (Plate **6**) shows nearly the same surface after the fifth cleavage is finished. The posterior surface is shown in Figures 46 (Plate **6**), 53, and 54 (Plate **7**); the animal pole, in Figure 45 (Plate **6**). Figure 48 shows a sagittal section, while a transverse section of a stage just later (looking toward the animal pole) is given in Figure 52.

The principal axis of the egg still coincides with its long axis, the animal pole lying at or near the micromere end, the vegetative pole at the macromere end (Fig. 48).

The egg now consists of (1) a single large cell, $d^{6.1}$, embedded within the other cells and appearing on the surface at the ventral end only (Plate **5**, Fig. 38), and (2) of thirty-one smaller cells, partly surrounding the larger cell $d^{6.1}$. One of these smaller cells, $d^{6.2}$, is a minute vesicle embedded between the cells $a^{5.1}$, $b^{5.1}$, and $d^{6.1}$ (Figs. 38 and 42).

The other thirty cells show a regular arrangement. The four quadrants may be distinguished as at the beginning of the fifth cleavage, each quadrant now containing eight cells, showing a characteristic arrangement. From ventral to dorsal we may now distinguish five layers. The first three layers contain each eight cells, two from each quadrant. In the quadrants *A*, *B*, and *C*, the two cells of a given layer are equal (Plate **6**, Fig. 47). In quadrant *D* there is great irregularity. The two cells of the ventral layer are extraordinarily unequal, constituting the large partly interior cell $d^{6.1}$, and the minute vesicle $d^{6.2}$, also enclosed within the other cells (Plate **5**, Fig. 38). In the second and third layers the two cells are likewise unequal, though less markedly so.

The fourth layer consists of a single large cell from each quadrant, that of quadrant *D* being the largest (Plate **6**, Fig. 45).

The fifth layer consists of four small cells at the dorsal pole of the egg (Fig. 45). The arrangement at the animal pole formed at the four-cell stage (Fig. 8) is still maintained. The quadrants *B* and *D* are in contact for a considerable distance, whereas *A* and *C* do not touch. In one of the points where three cells of different quadrants meet (in this case $b^{6.8}$, $c^{6.8}$, and $d^{6.8}$) lies the polar cell.

The first cells in which indications of cleavage are observed are again the large cells of the *D* quadrant, $d^{6.1}$ and $d^{6.3}$. Spindles are formed in these at about the same time (Plate **6**, Fig. 48). The processes taking place in the two cells differ, and must be considered separately.

In $d^{6.1}$, after the giving off of the small vesicle $d^{6.2}$, the nucleus very quickly enlarges to its original size. The aster begins to elongate at right angles to the position of the previous spindle (Plate **5**, Fig. 38). But at almost the same time a rotation takes place, and by the time the two asters are fully separated the line connecting them is seen to be nearly antero-posterior (Plate **5**, Fig. 42). The movement continues until the axis of the complex becomes exactly antero-posterior, and a spindle is formed *in precisely the same position as the spindle for the preceding cleavage.* This spindle is shown in the sagittal section, Figure 48. Its anterior end lies just ventrad of the small vesicle formed at the previous cleavage. Division now takes place, and a *second* small vesicle is given off to the point in the median plane lying just ventrad of the vesicle formed at the fifth cleavage. Figure 49 shows the process of formation of this vesicle, and Figure 50 shows the condition of affairs after the division is finished. In later stages the two vesicles are visible, lying beneath the cells of quadrants *A* and *B*, in the place where they were given off. (Compare Figure 50, Plate **6**, with Figures 56, Plate **7**, and 65, Plate **8**.)

Other changes occur at the same time in the large ventral cell. After the fifth cleavage the granular cloud in the cytoplasm gathered into the region of the anterior margin of the free surface of the cell (Plate **5**, Fig. 38). As the spindle for the sixth cleavage is formed, the cloud becomes concentrated over a small area, at a slight distance from the anterior margin of the cell (Plate **6**, Fig. 48). Then, as division takes place, the cloud moves up to the anterior margin, at the same time spreading out, and begins to pass beneath the cells of the quadrants A and B (Figs. 49 and 50). As the large nucleus moves away from the wall of the cell where the vesicle was formed, the granular cloud moves inward (dorsad) and spreads out between the nucleus of the large cell and the two vesicles (Figs. 51 and 52). The granules at this time have become very coarse and distinct.

Meantime, cleavage is taking place in the cell $d^{6.3}$. In this cell the changes occurring in the asters are peculiar.

Immediately after the preceding cleavage, the cell, having passed into the resting stage, has been pressed into an irregular wedge-shaped form by the processes occurring in the surrounding cells (Plate **5**, Fig. 37, surface view, and Fig. 38, section, from the same egg). The cell has become very narrow at the level at which the nucleus lies, so that, apparently, there is not room for the asters to separate at right angles to the foregoing spindle. The nucleus is pressed closely against the ventral wall of the cell (Fig. 38), and the aster begins to extend obliquely along the dorsal side of it, between the nucleus and the dorsal wall of the cell. When the aster has become completely divided and the products are on opposite sides of the nucleus, their common axis is already in the same direction as the axis of the spindle at the previous division. The same result is obtained as in the rotation at the fourth cleavage, though in a different manner. *But the final position is not yet reached.*

As now situated, the asters lie in the long axis of the much elongated cell (Plate **6**, Fig. 46). As the active condition preparatory to division comes on, the cell withdraws its deeper parts (shown in Plate **5**, Fig. 38), and its dorso-ventral dimension increases. Accompanying this change is a rotation of the nuclear complex, from a position with the axis in the greatest dimension of the cell, to a position with axis in the shortest dimension. This change is shown in progress in Plate **7**, Fig. 53. A later stage is shown in Figure 54; here the spindle is completely formed. The dorso-ventral axis of the cell has greatly increased, but is still distinctly less than the width of the cell at right angles to the spindle. The cytoplasm has become grouped symmetrically about the spindle, with the latter in its *short* axis. A longitudinal section of the same egg is shown in Plate **6**, Fig. 48; the completed cleavage ($d^{7.5}$ and $d^{7.6}$) is shown in section in Figure 50, and from the surface in Plate **7**, Fig. 57. The cleavage is unequal, the ventral cell being much the smaller.

Cleavage in the other cells of this generation takes place in a sequence that is complicated by various factors, so that the account will be clearer if the divisions of the cells are described in connection with their relative positions in the egg, reserving a discussion of the order of cleavage till the end. The divisions will be taken up according to the layers of cells, beginning with the ventral layer.

First or Ventral Layer, consisting of the eight cells, $a^{6.1}-d^{6.1}$ and $a^{6.2}-d^{6.2}$.

The cleavage of $d^{6.1}$ has been described. The small vesicle $d^{6.2}$ does not divide farther. The other cells of this layer divide equatorially into cells of equal size. Two of the spindles leading to this cleavage are shown in Figure 56 (Plate **7**), and the completed cleavage in Figure 61. The resulting fourteen cells are $a^{7.1}-d^{7.1}$, $a^{7.2}-d^{7.2}$, $a^{7.3}-c^{7.3}$, and $a^{7.4}-c^{7.4}$.

Second Layer, containing the cells $a^{6.3}-d^{6.3}$ and $a^{6.4}-d^{6.4}$.

The cleavage of $d^{6.3}$ has been described; it is equatorial and unequal. The remainder of the cells also divide by equatorial furrows, but the products are equal in size. One of the spindles is shown in $c^{6.4}$, in Figure 47 (Plate **6**), and in Figure 55 (Plate **7**) the nearly completed cleavage; the nuclei in all but the products of $c^{6.3}$ are still connected by interzonal filaments. The same condition of the cell $d^{6.4}$ is shown in Figure 57 (the products being $d^{7.7}$ and $d^{7.8}$). In all of these cells, except $d^{6.4}$, the spindles lie at first in the shorter axes of the cells, as indicated in Figure 47 (Plate **6**); but as the karyokinetic processes progress, the cells elongate in the direction of the spindles until the axes in which the spindles lie are the longer.

The products of this division are $a^{7.5}-d^{7.5}$, $a^{7.6}-d^{7.6}$, $a^{7.7}-d^{7.7}$, and $a^{7.8}-d^{7.8}$.

Third Layer, containing the eight cells $a^{6.5}-d^{6.5}$ and $a^{6.6}-d^{6.6}$.

In all these cells the division is meridional, not equatorial, as in the cells of the first and second layers. One of the spindles ($b^{6.5}$) is shown in Figure 55 (Plate **7**). The cleavage in $d^{6.5}$ and $d^{6.6}$ is shown in Figure 57. In this figure, $d^{6.5}$ has already divided into $d^{7.9}$ and $d^{7.10}$, the nuclei of which are still connected by interzonal filaments. In $d^{6.6}$ the spindle is still present. The completed division in the anterior part of the egg is shown in Figure 61. The cleavage is equal in all the cells of this layer except $d^{6.6}$ and $c^{6.6}$; in these it is unequal. The unequal cleavage of $c^{6.6}$ is shown in Figure 58, and the same figure shows the unequal products ($d^{7.11}$ and $d^{7.12}$) resulting from the division of $d^{6.6}$.

By the division of the third layer a band of sixteen cells is produced, extending completely around the embryo. The cells composing the band are $a^{7.9}-d^{7.9}$, $a^{7.10}-d^{7.10}$, $a^{7.11}-d^{7.11}$, and $a^{7.12}-d^{7.12}$.

Fourth Layer, containing the four cells $a^{6.7}-d^{6.7}$.

In these cells the cleavage is meridional, as in the third layer, and in every case equal. The spindles in $b^{6.7}$ and $c^{6.7}$ are shown in Figure 47 (Plate **6**).

The eight cells resulting from this cleavage are $a^{7.13}-d^{7.13}$ and $a^{7.14}-d^{7.14}$

Fifth Layer, containing the four cells $a^{6.8}-d^{6.8}$, situated at the animal pole of the egg.

The four small cells at the animal pole of the egg divide equatorially. The spindle of $d^{6.8}$ is shown in Figure 59 (Plate **7**); and of $a^{6.8}$ in Figure 60. The cleavage products are very unequal; the dorsal cells so formed are very minute, so that the distinction between cell body and nucleus cannot be observed, and the cells cannot be distinguished from the polar cell lying in the same region. Figure 60 shows the cleavage at the animal pole completed except in the cell $a^{6.8}$. A group of three small vesicles, representing the dorsal cleavage products of the cells $b^{6.8}-d^{6.8}$, lie at the animal pole, surrounded by the four larger cells, — one of which is the undivided cell $a^{6.8}$, while the others are the ventral cleavage products of $b^{6.8}-d^{6.8}$.

The eight cells thus produced are $a^{7.15}-d^{7.15}$ and $a^{7.16}-d^{7.16}$

This, the sixth, cleavage may be tabulated as follows: —

Layer.	Cells.	Direction of Cleavage.	Nature of Cleavage.	Product.
First, or Ventral	(a, b, c, d) $^{6.1}$ and $^{6.2}$	Equatorial (except $d^{6.1}$)	Equal (exc. $d^{6.1}$)	$(a-d)$ $^{7.1}$ and $^{7.2}$, $(a-c)$ $^{7.3}$ and $^{7.4}$ [and $d^{6.2}$].
Second	(a, b, c, d) $^{6.3}$ and $^{6.4}$	Equatorial	Equal (exc. $d^{6.3}$)	$(a-d)$ $^{7.5}$ and $^{7.6}$; $(a-d)$ $^{7.7}$ and $^{7.8}$.
Third	(a, b, c, d) $^{6.5}$ and $^{6.6}$	Meridional	Equal (exc. $c^{6.6}$, $d^{6.6}$)	$(a-d)$ $^{7.9}$ and $^{7.10}$; $(a-d)$ $^{7.11}$ and $^{7.12}$.
Fourth	(a, b, c, d) $^{6.7}$	Meridional	Equal	$(a-d)$ $^{7.13}$ and $^{7.14}$.
Fifth	(a, b, c, d) $^{6.8}$	Equatorial	Unequal	$(a-d)$ $^{7.15}$ and $^{7.16}$.

Since the minute cell $d^{6.2}$ does not divide, we thus have produced sixty-three cells instead of the typical number, sixty-four. Such a stage does not, however, have an actual existence, since some of the divisions belonging to the seventh cleavage have taken place before all these cleavages are finished.

Figure 61 (Plate **7**) shows the anterior surface of the egg at the end of the sixth cleavage, Figure 58 the posterior surface, Figure 60 the dorsal pole, and Figure 63 (Plate **8**) the ventral pole.

Sequence of the Sixth Cleavage. — The order in which the cells divide is, as I have already stated, now complicated by several factors.

(1) The divisions of the first four quadrants of the egg (at the third cleavage) were not synchronous, but followed in the order *D, C, B, A.* Other conditions remaining the same, — that is, with equal intervals between the ensuing cleavages, — the same order would obtain in the later stages.

(2) As discussed on page 35, the sequence becomes modified during the fifth cleavage, so that the cells in any given quadrant divide in nearly or quite the order of size of the blastomeres, beginning with the largest. In the three quadrants *A, B,* and *C,* the order is (the ventral cell in each case being considered number one) 4, 2, 1, 3, while in the quadrant *D* the order is 1, 2, 4, 3. This order would naturally reappear in the sixth cleavage, other conditions remaining the same.

Both the above factors do influence the fifth cleavage, but with still further complications. The first factor appears in the fact, that in any given layer the general order of cleavage of the component cells is *D, C, B, A.*

The second factor is shown by considering the cleavage of a single quadrant, as *D.* The order of cleavage for the large left hand cells of this quadrant is as follows, naming the layers from ventral to dorsal: 1, 2, $\left\{{}^{3}_{4}\right.$, — nearly or quite the same as at the last cleavage.

But a third factor appears in comparing the large left hand cells $d^{6.3}$ and $d^{6.5}$ of the *D* quadrant with their small right hand sister cells $d^{6.4}$ and $d^{6.6}$ (Plate **5**, Fig. 37, Plate **6**, Fig. 46, Plate **7**, Figs. 54 and 57). *The large cells of each pair divide first,* though the age of the two, being sister cells, is exactly the same.

Similar relations may be shown for the other quadrants. Two facts are worthy of particular notice. (1) The large cells $a^{6.7} - c^{6.7}$ divide first of all the cells in the quadrants *A, B,* and *C,* — and long before the small cells $a^{6.8} - c^{6.8}$, which are of exactly the same age. (2) There are some variations which cannot be brought into relation with any of the factors mentioned. Thus, Figure 56 (Plate **7**) shows that the cell $b^{6.1}$ is dividing before the cells $c^{6.1}$ and $b^{6.2}$, though the cells are apparently of the same size, and from the sequence of preceding cleavages the cell $c^{6.1}$ would be expected to divide first. However, such variations *may* be correlated with differences in the size of the cells, since it is impossible to calculate precisely the volume of cells which have such irregular forms, and are subjected to varying conditions with the changing positions of the surrounding cells.

Certain general facts appear from the preceding discussion of the sixth cleavage. (Compare the table of this cleavage, on page 39.)

(1) Every cell of any quadrant cleaves with its spindle in the same direction as the corresponding cell of any other quadrant (except the large interior cell $d^{6.1}$).

The cleavage of a single typical quadrant up to this time is shown in the annexed diagram (Diagram I.).

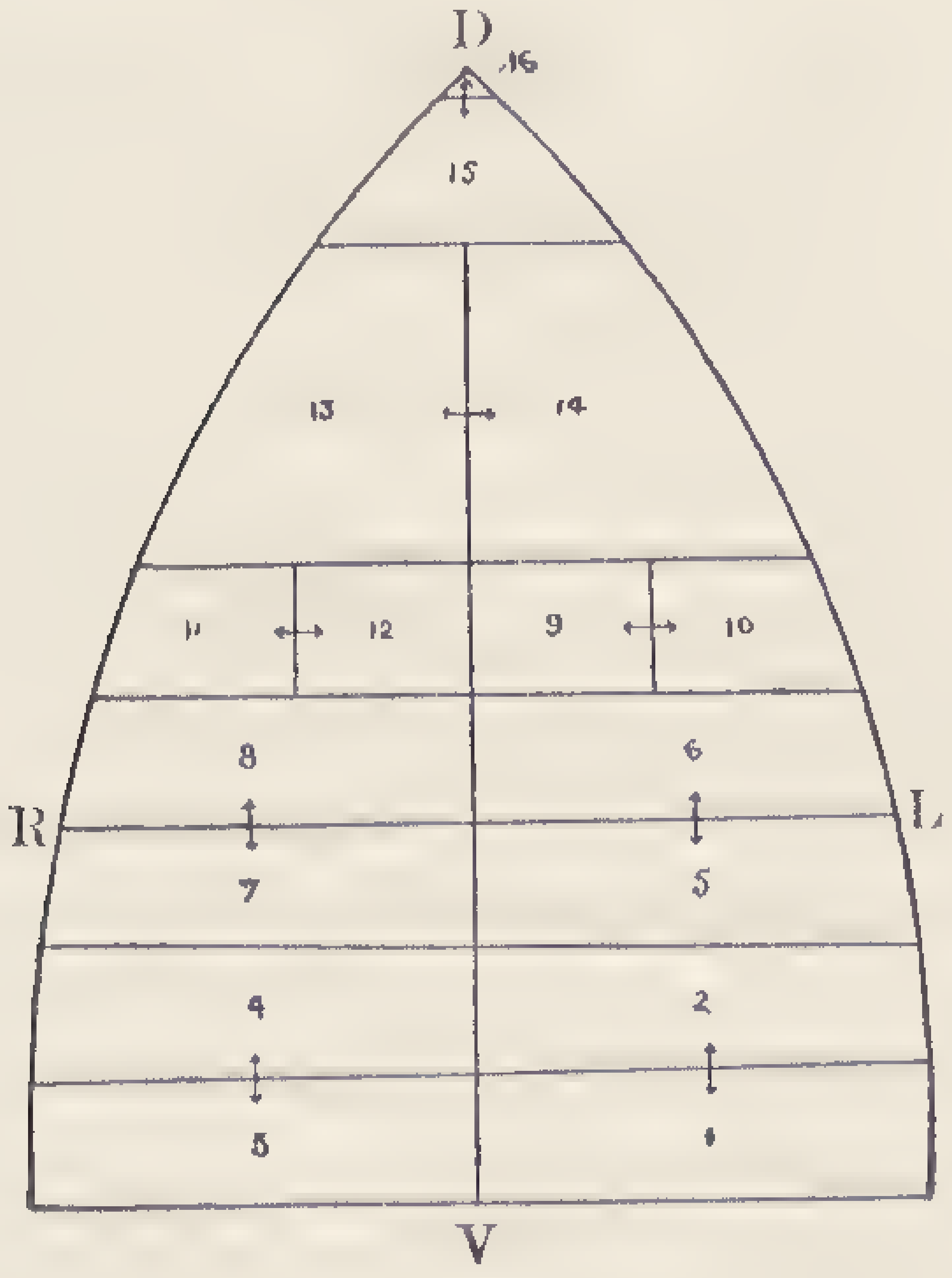

Diagram I.

Diagram of quadrant *A*, *B*, or *C* in the seventh generation. Only the second exponent designating the cells appears in the diagram, the first being in all cases 7. Thus, the cell labelled 5 represents $(a, b, \text{or } c)^{7.5}$. The arrows connect cells of common origin, and show the direction of the spindles at the preceding division. R signifies right; L, left; D, dorsal; V, ventral; according to the plan of orientation explained at page **14**.

(2) All the cells in any layer (series of cells occupying the same relative position between the dorsal and ventral poles of the egg) cleave with spindles in the same direction (except $d^{6.1}$).

(3) All the cleavages are equal except in the dorsal (fifth) layer, and in $d^{6.1}$, $c^{6.6}$, and $d^{6.6}$.

(4) There is a tendency for the largest cells to cleave fastest. The minute cell $d^{6.2}$ does not cleave at all.

(5) The cell $d^{6.1}$ cleaves in such a manner as to form a marked exception to the method followed by the other cells. Its cleavage is very

unequal, while all the other cells in the ventral layer cleave equally. Also, the spindle does not lie in the same direction as in the other cells of this layer.

The exceptional nature of the division in $d^{6.1}$ evidently demands explanation. The regularity of the cleavage in the other cells of the egg is such that some special condition must be correlated with the markedly differing division of this cell. The cleavage of $d^{6.1}$ differs from that of the other cells of the ventral layer in the following points. (1) Its cleavage is very unequal, while the cleavage of the other cells is equal. (2) The spindle for the sixth cleavage in $d^{6.1}$ lies in almost exactly the same position as did the spindle for the preceding cleavage, whereas in the other cells of the ventral layer the spindle for the sixth cleavage is at right angles to the position of the spindle for the fifth cleavage.

As to the first point, — that of unequal division, — no special correlating factor for *this* particular case seems necessary, since in many cases in this and preceding cleavages the cells varied as to the equality of the division, though alike in other respects. Thus, in the fifth cleavage, the three ventral cells of the *D* quadrant cleft unequally, though in all the other quadrants the division was equal; and in this sixth cleavage the cells $c^{6.6}$ and $d^{6.6}$ divide unequally, though in all the other cells of the same layer the division is equal.

But the second point is totally exceptional in the cleavage up to this time. The axial relations of the cells appear to be so distinct and constant, and there is such uniformity in the positions of spindles at a given cleavage among cells of similar origin and relative position, that one must look for some other marked difference in the cell $d^{6.1}$ that might occasion this change of axis.

In what respects does the cell $d^{6.1}$ differ from the other cells of the egg? (1) In its greater size; (2) in its position.

(1) The greater size evidently has nothing to do with the different direction of cleavage, since the same disparity in size was present in earlier cleavages, yet this cell divided in the same direction as did the other ventral cells.

(2) The change of position is such as to bring about a fundamental change in the relations of the cell to the egg as a whole and to the other cells. Previously the cell $d^{6.1}$ formed the posterior cell of the ventral layer. At the time of the sixth cleavage the cell has moved toward the interior of the egg and its posterior surface is covered as far ventrad as its anterior surface. The cell is now *central*, and surrounded

on all sides by other cells. It thus occupies a position in the egg which is fundamentally different from that occupied by any other cell. Correlated with this fundamentally different position, the cell acquires a fundamentally different method of division.

It is impossible to say whether any particular feature of the different position of the cell is the essential one in bringing about this altered method of cleavage. As the cell moves inward, it very probably accomplishes a partial rotation (see below); if the axes of the cell are definite, and *determined within the cell alone*, then this rotation would cause a change in the position of the axes of the cell $d^{6.1}$ in comparison with the axes of the other cells, and a different direction of the spindle would result. But any such explanation is hypothetical.

During the later stages of the sixth cleavage, the process of *gastrulation* has made much progress. At the end of the fifth cleavage the large ventral cell $d^{6.1}$ had already moved some distance toward the interior of the egg (Plate **5**, Fig. 38). As the cells $d^{6.3}$ and $d^{6.5}$ now withdraw successively their deeper parts and increase their surface extension during division, they push ventrad, displacing the posterior part of the ventral cell (now $d^{7.1}$). (Compare Plate **6**, Figs. 48, 50, and 51.) The large cell therefore pushes dorsad into the interior of the egg, occupying the space made vacant by the ventral extension of the other cells. Soon after, the anterior cells, belonging to quadrants *A*, *B*, and *C* also enter upon the karyokinetic process, and in so doing likewise push ventrad (Plate **8**, Fig. 64) at the same time vacating of course a portion of the space before occupied by them near the animal pole. The cell $d^{7.1}$ therefore continues to move dorsad, so that at the end of this cleavage it is almost completely enclosed (Fig. 65).

During this inward movement of the cell $d^{7.1}$, the cloud of granules previously described changes its position still further. We had traced it, after the sixth cleavage, until it occupied a position between the nucleus of $d^{7.1}$ and the two vesicles formed at the fifth and sixth cleavages (Plate **6**, Fig. 51). As gastrulation continues, the cloud of granules migrates still further dorsad, and later even crosses the dorso-ventral axis, so as to lie posterior to it, surrounding the dorsal aster at the next division of $d^{7.1}$ (Plate **8**, Fig 64).

This movement of the cloud of granules *possibly* gives the key to the change of axis of division in the cell $d^{7.1}$. When the *posterior* cells extend ventrad during division, as previously described (Plate **6**, Figs. 48 and 51), they push against the posterior side of the cell $d^{6.1}$, thus displacing the cell inward. But such an impulse from one side only would naturally, if the cell be a body not entirely fluid, give it a rotary motion. As a result of the partial rotation, the cloud of granules is now found underneath the anterior cells (Plate **6**, Fig. 50). When the *anterior* cells extend ventrad, the form of $d^{7.1}$ is so changed by its change of position that the cells do not *push against* it, but glide over it, at the same time vacating a part of the dorsal region of the egg (Plate **8**, Fig. 64). Therefore the cell $d^{7.1}$, as it moves inward, continues its rotation in the same direction as before, and the cloud of granules is brought to the dorsal end of the cell, and even farther.

Such a rotation would explain clearly the peculiar position of the spindle in $d^{6.1}$ at the sixth cleavage. To agree with the other cells of the ventral layer, the spindle would have to take a dorso-ventral position. (Compare Fig. 55, Plate **7**.) But a rotation from posterior to anterior just before this cleavage would bring the spindle into an antero-posterior position, such as actually occurs.

However, the explanation is not very satisfactory, for several reasons. (1) We have previously seen that the granules forming the granular cloud *do* move *within the cytoplasm* of the cell, so that this change of position of the granular mass may be due to simple migration through the protoplasm of the cell. (2) A study of the movements of the asters after the fifth and sixth cleavages does not give evidence of any such rotation. (See the account of the movements of the asters before the sixth cleavage, page 36, and before the seventh cleavage, page 54.) (3) Such a rotation explains the position of the spindle at the sixth cleavage only, — while all the later cleavages of the inner cells are likewise out of relation to the divisions of the outer cells. (4) The explanation assumes that the position of the axes of cleavage is definite, and *determined within the cell itself*, so that, if the cell rotates, the axis rotates with it, — which is not proved.

The only conclusion in which we are entirely justified is therefore merely this, that as the relation of the cell $d^{6.1}$ to the other cells and to the egg as a whole becomes fundamentally changed, the method of division likewise becomes fundamentally changed.

At the close of the processes thus far described, the egg has evidently passed into the "gastrula stage" proper (Plate **8**, Fig. 64). The blastopore is still large and lies at the macromere end of the egg. It is surrounded at first by eight cells, two belonging to each of the four original quadrants of the egg (Plate **7**, Fig. 56). Later, as the sixth cleavage is entirely finished, one of the cells ($c^{7.1}$) becomes displaced and is shut out from the margin of the blastopore (Plate **8**, Fig. 63), which is itself diminished in size. The interior of the gastrula is occupied by the large cell $d^{7.1}$ and the two minute vesicles $d^{6.2}$ and $d^{7.2}$ (Fig. 64). The interior cells are surrounded by a single layer of outer cells, except at the animal pole of the egg, where the small dorsal cells do not reach to the cells within the gastrula, but lie on the surface, making here a two-layered region. This region remains two-layered as long as it is possible to trace the history of the animal pole of the egg.

The three inner cells $d^{7.1}$, $d^{6.2}$, and $d^{7.2}$, with the products of the former, may henceforth be called the *entoderm*, the outer layer the *ectoderm.*

Seventh and Later Cleavages.

I have followed the cleavage through another generation, and, for parts of the egg, much farther. It becomes impracticable, however, to describe the cleavage according to the layers or series in which it takes place, as has been done up to this stage, owing to the complicated succession of the divisions in the different cells, and to the great changes in position taking place while the cleavages are in progress. I shall therefore now describe the processes in the general order in which they occur, and in so doing I shall consider separately (1) the ectoderm, and (2) the entoderm.

THE ECTODERM.

In discussing the changes taking place in the ectoderm, it will be well to distinguish for convenience of description two regions: (1) the (left) posterior part of the ectoderm, derived from the quadrant *D;* (2) the anterior and right lateral portions of the ectoderm, derived from the quadrants *A*, *B*, and *C*. While the phenomena occurring in all of these regions are reducible to the same general scheme, so far as the method of cleavage is concerned, the irregularity in the size of the blastomeres forming quadrant *D*, their earlier cleavage as compared with the other quadrants, and the fact that some of the cells have passed inward to form the entoderm, give this region a peculiar and somewhat irregular character, which makes it convenient to discuss it separately.

(1) *The Quadrant D.* — The entoderm cells belong genetically to this quadrant, but they will be considered later.

In order to understand the conditions in quadrant *D*, and to see their relations with the arrangement in the other quadrants, it will be well to emphasize certain features of the last two cleavages.

At the fifth cleavage, as previously described, the three ventral cells of quadrant *D* divided by meridional planes into unequal portions, the left derivative being in every case larger (Plate **4**, Fig. 33, and Plate **5**, Fig. 37). The single dorsal cell divided equatorially into a small dorsal and a large ventral part. There is appended a diagram of the quadrant *D* after this cleavage (Diagram II.). If the septa in the three ventral cells were moved to the middle of the cells, the diagram would represent the condition in any one of the other three quadrants.

At the next division (sixth) the two ventral cells $d^{6.1}$ and $d^{6.2}$ have passed inward, becoming the entoderm, so that we may omit them from the present discussion. Of the other cells, the ventral pair ($d^{6.3}$ and $d^{6.4}$) divide equatorially, $d^{6.3}$ unequally, $d^{6.4}$ equally (see Plate **7**, Figs. 57 and 58). The two next layers divide meridionally (Fig. 57), the cell $d^{6.6}$ unequally, the others equally. The dorsal cell divides equatorially and unequally. Diagram III. shows the ectodermal part of this quadrant at the end of the sixth cleavage. The actual condition in the egg at this period is shown in Figure 58, and at a slightly earlier stage in Figure 57.

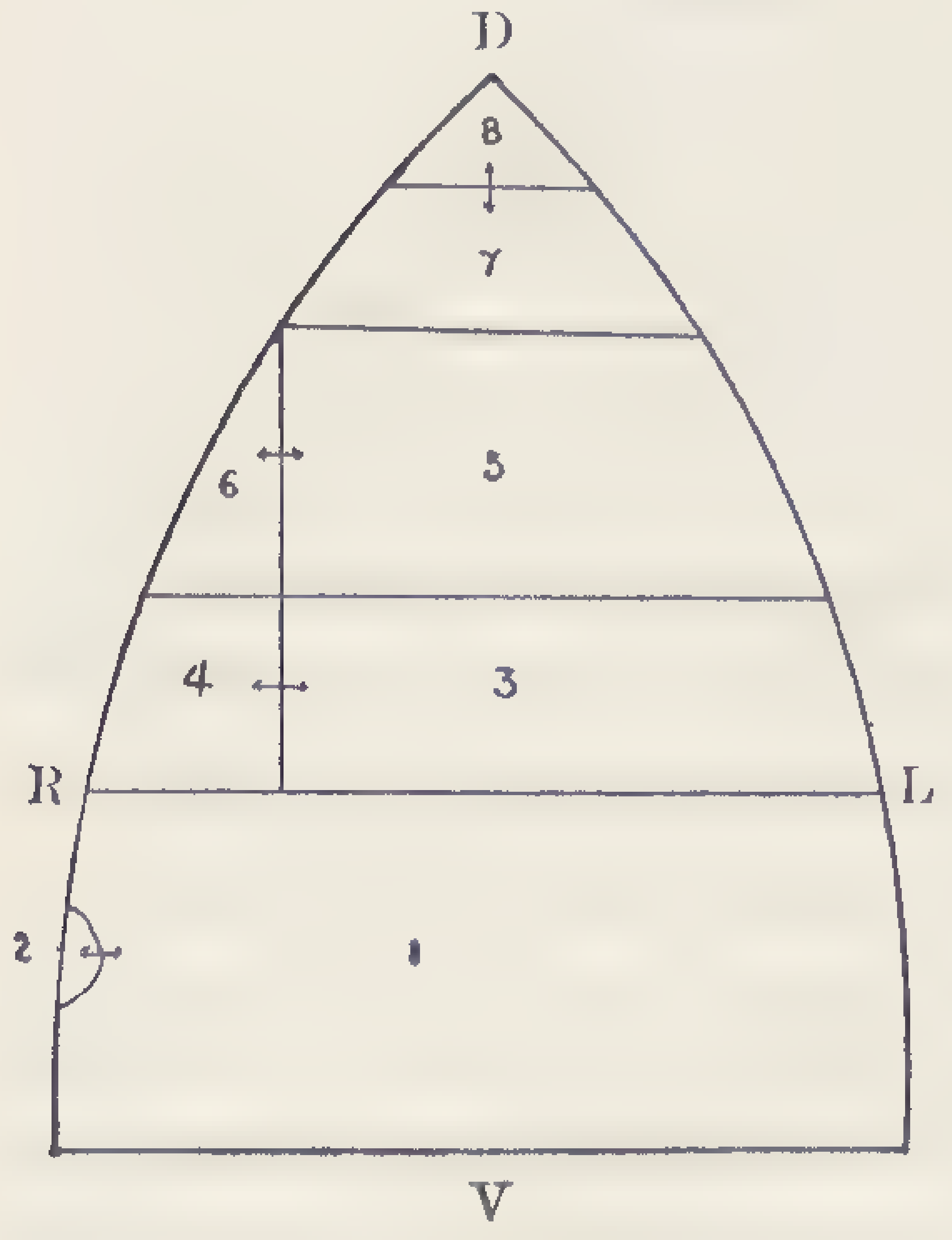

DIAGRAM II.

Diagram of quadrant *D* in the sixth generation. Only the second exponent designating the cells appears in the diagram, the first being in all cases 6.

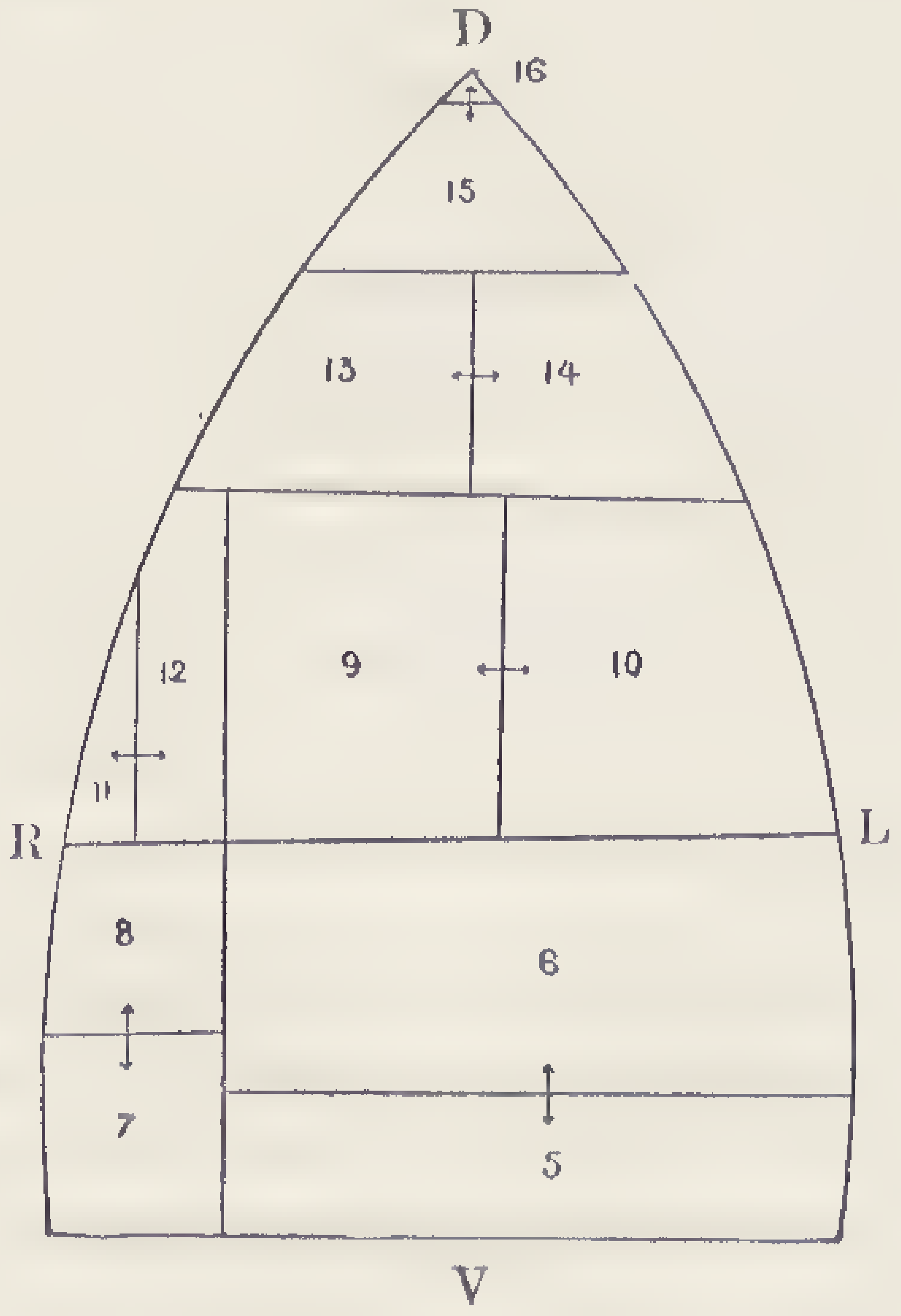

DIAGRAM III.

Quadrant *D* in the seventh generation. Only the second exponent is expressed, the first being in all cases 7.

The arrows connect cells of common origin, and show the direction of the spindles at the preceding division. R signifies right; L, left; D, dorsal; V, ventral.

Comparison of Diagram III. with the type diagram for the other three quadrants given on page 41, shows that the *directions* of the cell walls are the same in both, the inequality in the size of the cell in the quadrant *D* being the only difference.

In the *seventh cleavage*, the spindle appears first in the cell $d^{7.6}$, as shown in Figure 58, and the cell is divided meridionally into two equal cells, $d^{8.11}$ and $d^{8.12}$. The finished division is shown in Plate **8**, Figs. 66, 67, and 68. The plane separating these two cells is the median dorso-ventral plane of the embryo, as will be shown later.

Shortly after this division is completed, spindles appear in $d^{7.5}$, $d^{7.9}$, $d^{7.10}$, $d^{7.13}$, and $d^{7.14}$, as shown in Figure 66. In $d^{7.5}$ the spindle is dorso-ventral, hence lying in the shorter axis of the cell; the cell extends and divides into two equal dorsal and ventral parts, $d^{8.9}$ and $d^{8.10}$ (Figs. 67 and 68). The greater surface extension of $d^{8.9}$ in Figure 67 is due to its being spread out in a thin layer over the surface of the entoderm cell.

In $d^{7.9}$ and $d^{7.10}$ (Fig. 66) the spindles are also dorso-ventral in position, and the cells divide equatorially into equal parts, $d^{8.17}$, $d^{8.18}$, $d^{8.19}$, and $d^{8.20}$ (Figs. 67 and 68).

In $d^{7.13}$ and $d^{7.14}$ the spindles lie at right angles to those just described, and the cells divide meridionally and equally, forming $d^{8.25}$, $d^{8.26}$, $d^{8.27}$, and $d^{8.28}$.

Figure 67 is a view of this region after these cleavages are finished. As shown in this figure, a certain amount of shifting has taken place during cleavage, by which the cell $d^{7.7}$ has been excluded from its share in the boundary of the blastopore. As the cells divide, they withdraw their interior parts and extend in the direction of the spindle, as has been minutely described for other cleavages; in this way the dorso-ventral extent of quadrant *D* has been greatly increased. As a result the blastopore has been nearly closed (Fig. 63), and at the opposite end the animal pole has been pushed beyond the micromere end of the egg to its anterior side (Fig. 65).

Thus far the six larger left hand cells have divided, leaving six smaller cells (at the right and at the dorsal pole) undivided (Fig. 67).

Next, as shown in this figure, the cells $d^{7.7}$ and $d^{7.8}$ form spindles and divide. Each cleaves in the same manner as its larger companion cell has done, $d^{7.7}$ equatorially, $d^{7.8}$ meridionally. The products, $d^{8.13}$, $d^{8.14}$, $d^{8.15}$, and $d^{8.16}$, are shown in Figure 68 (compare Diagram IV.).

Next $d^{7.12}$ cleaves equatorially, like its companion cells $d^{7.9}$ and $d^{7.10}$. In $d^{7.12}$ the division is unequal, the ventral product, $d^{8.23}$, being much the smaller (Fig. 68).

The cleavage of $d^{7.11}$ occurs much later, and is likewise equatorial. I have not thought it necessary to introduce a special figure to show the cleavage of this minute cell.

In $d^{7.15}$ the cleavage also takes place late; it is meridional and equal. The resulting cells, $d^{8.29}$ and $d^{8.30}$, are shown in Figure 72 (Plate **9**). The minute dorsal cell $d^{7.16}$ does not divide.

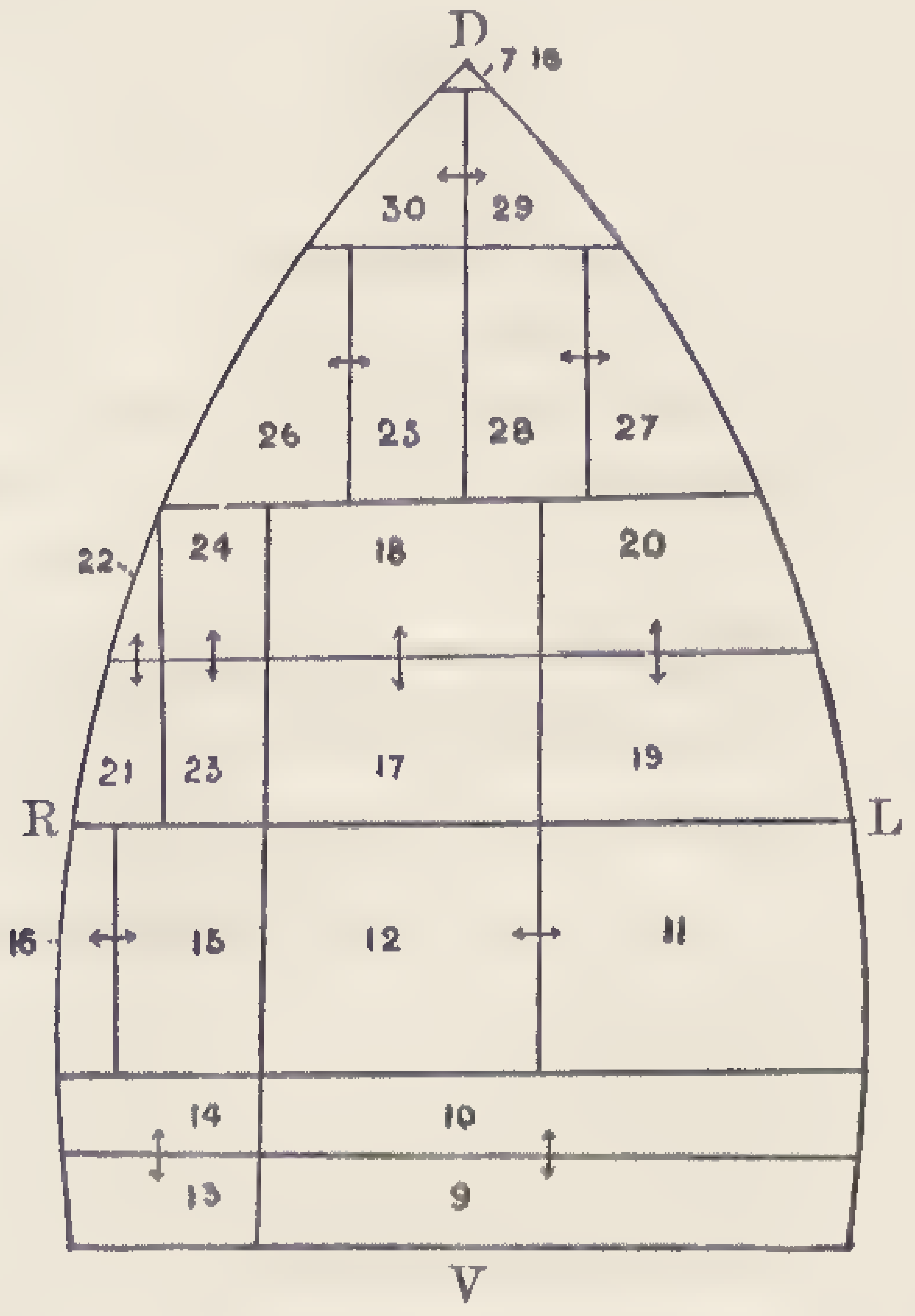

DIAGRAM IV.

Quadrant *D* in the eighth generation, — except the dorsal cell, $d^{7.16}$, which does not divide farther. In the other cells only the second exponent is expressed, the first being in all cases 8. The arrows connect cells of common origin, and show the direction of the spindles at the preceding cleavage.

R signifies right; L, left; D, dorsal; V, ventral.

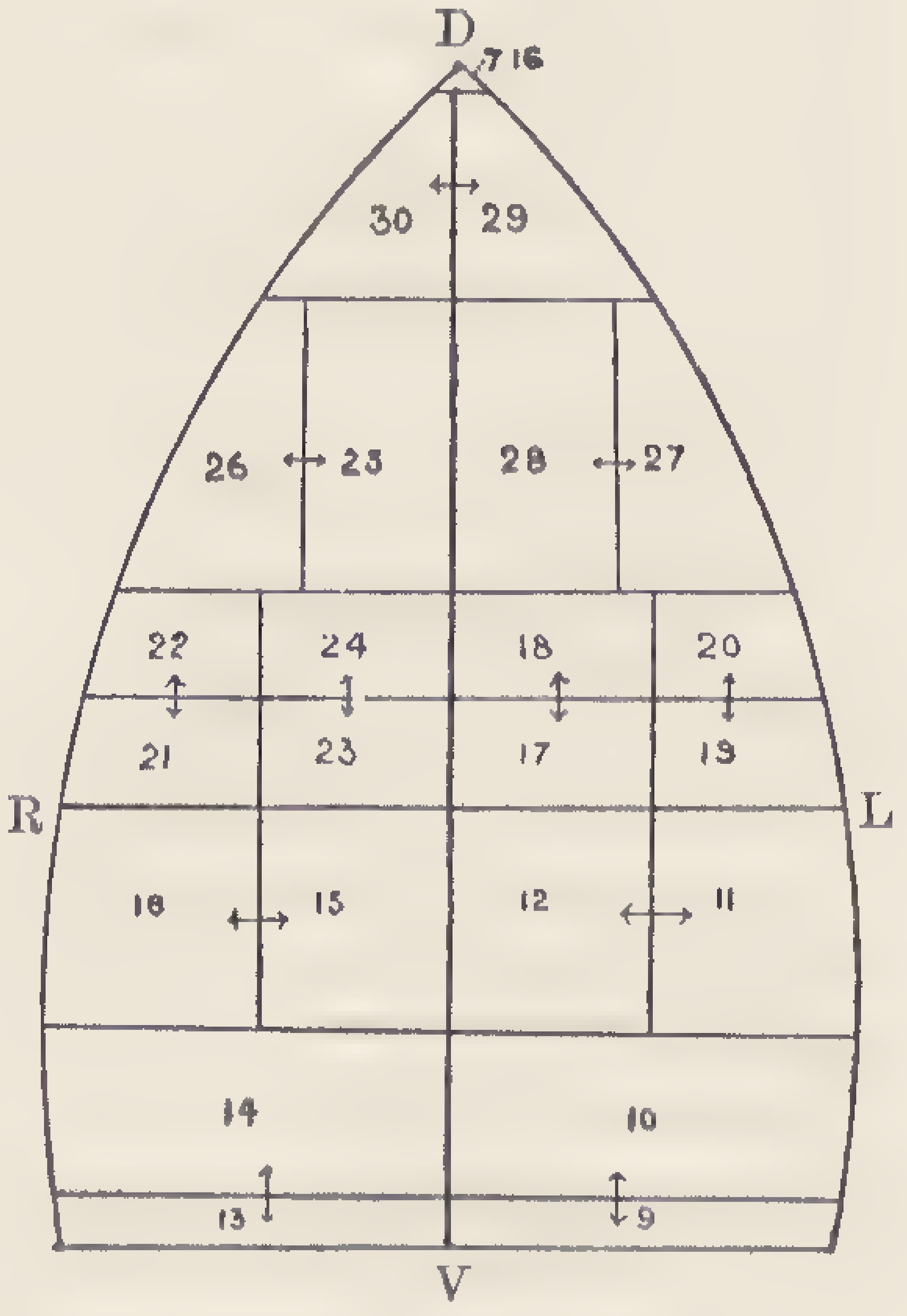

DIAGRAM V.

Diagram of quadrant *A*, *B*, or *C*, in the eighth generation. Only the second exponent of the cells appears in the diagram, the first being 8. The small dorsal cell, $(a, b, \text{or } c)^{7.16}$, does not divide, remaining thus in the seventh generation. The arrows connect cells of common origin, and show the direction of the spindle at the preceding division.

R signifies right; L, left; D, dorsal; V, ventral.

There are thus 23 cells in the ectodermal part of the quadrant *D* at the end of the seventh cleavage. A diagram of this stage is annexed (Diagram IV.). Nearly this stage is represented in Figure 68.

(2) *The Quadrants A, B, and C.* — Owing to the regularity in the size of the cells in these quadrants, and the fact that they are purely ectodermal, the conditions observed are fairly simple as compared with

those in quadrant *D*. Figure 61 (Plate **7**) shows the anterior surface of the egg at the end of the sixth cleavage, and a diagram of a single quadrant at this stage was given on page 41. Comparing either the figure or the diagram with the scheme (Diagram III.) given on page 46 for the quadrant *D* at the same stage, the arrangement is seen to be the same except in two respects. (1) The cells belonging to the same lateral series are equal in *A*, *B*, and *C*, unequal in *D*. (2) Four ventral ectodermal cells additional are present in each of the quadrants *A*, *B*, and *C*; these are represented in *D* by the cells which have passed inward to form the entoderm.

As shown by the spindles in Figure 61, the first cells to divide are $a^{7.13}-c^{7.13}$ and $a^{7.14}-c^{7.14}$. The cleavage is meridional and equal. The resulting cells are $a^{8.25}-c^{8.25}$, $a^{8.26}-c^{8.26}$, $a^{8.27}-c^{8.27}$, and $a^{8.28}-c^{8.28}$. They are shown in Figures 72 and 75 (Plate **9**). (Compare Diagram V.)

Next follows the division of the six cells, $a^{7.6}-c^{7.6}$ and $a^{7.8}-c^{7.8}$, which together form part of a transverse girdle, surrounding the egg. The cleavage is meridional and equal. The resulting 12 cells, $a^{8.11}-c^{8.11}$, $a^{8.12}-c^{8.12}$, $a^{8.15}-c^{8.15}$, and $a^{8.16}-c^{8.16}$, forming as before a transverse girdle, are shown in Plate **8**, Figs. 69 and 70, and Plate **9**, Fig. 71.

Next ensues the cleavage of the transverse row containing the six cells, $a^{7.5}-c^{7.5}$ and $a^{7.7}-c^{7.7}$. These cells, as shown in Figure 61 (Plate **7**), are much flattened dorso-ventrally, and are of exactly the same form as the cells last discussed, which lie immediately dorsad of them. Moreover, each cell in this row corresponds in origin to a cell of the row last described, the two rows having been derived from the equatorial division of a previously existing transverse row, as shown in Figure 55. If mechanical conditions are decisive in determining the direction of the cleavage, these two rows should cleave in the same manner, i. e. both meridionally. Nevertheless, as shown in Figure 69 (Plate **8**), while the dorsal of the two rows divides meridionally, the cells of this ventral row all cleave equatorially. The axis of the cell in which the spindle lies is about half as long as the axis which is at right angles to it. The cells elongate in the direction of the spindles, and a very unequal division ensues. The ventral products are minute, while the dorsal ones are nearly equal in size to the mother cells. Figure 75 (Plate **9**) shows the anterior surface of the egg after this cleavage. The twelve cells produced are $a^{8.9}-c^{8.9}$, $a^{8.10}-c^{8.10}$, $a^{8.13}-c^{8.13}$, and $a^{8.14}-c^{8.14}$.

The division of the band of twelve small cells composed of $a^{7.9}-c^{7.9}$, $a^{7.10}-c^{7.10}$, $a^{7.11}-c^{7.11}$, and $a^{7.12}-c^{7.12}$, and shown in Figure 61 (Plate **7**), follows somewhat later. The cleavage is equatorial and the spindles lie in the short axes of the cells, as shown in Figure 75 (Plate **9**). Just before division the cells elongate until the axis in which the spindle lies is longest, as shown in the cell $b^{7\cdot12}$ of the figure last mentioned.

The cells $a^{7\cdot15}-c^{7\cdot15}$, near the animal pole, cleave meridionally. The spindle in $a^{7\cdot15}$, and the cells $b^{8\cdot29}$ and $c^{8\cdot29}$, $b^{8\cdot30}$ and $c^{8\cdot30}$, are shown in Figure 72 (Plate **9**). The minute cells $a^{7\cdot16}-c^{7\cdot16}$, like $d^{7\cdot16}$, do not cleave further. The arrangement at the animal pole is now very irregular, and owing to this fact and the minuteness of the cells produced at the last cleavages it is very difficult to be certain of the exact origin of any given cell, though the origin of the *group* cannot of course be doubtful.

We have now accounted for all the cells of these quadrants except the four ventral cells of each quadrant, $a^{7\cdot1}-c^{7\cdot1}$, $a^{7\cdot2}-c^{7\cdot2}$, $a^{7\cdot3}-c^{7\cdot3}$, and $a^{7\cdot4}-c^{7\cdot4}$. These correspond in origin to the entoderm cells of quadrant *D*, and they do not cleave further until they are partly or entirely enclosed within the embryo, as will be shown later.

A diagram of that part of any one of the quadrants *A*, *B*, and *C* corresponding to the ectodermal part of quadrant *D*, and showing the conditions at the end of the seventh cleavage, is given on page 48 (Diagram V.). A comparison of this diagram with that for the corresponding stage of the quadrant *D* (Diagram IV. on the same page) shows that the direction of the cleavage planes, and hence of the spindles, is the same throughout in all cells of corresponding position, though there are many differences as to the equality or inequality of the cleavage products.

The general facts which may be deduced from the foregoing study of the seventh cleavage in the ectodermal parts of the egg are similar to those drawn from a study of the sixth cleavage, page 41.

(1) Every cell of any *quadrant* divides with its spindle in the same direction as the corresponding cell of any other quadrant.

(2) All the cells in any *layer* cleave with spindles in the same direction (in spite of great differences in the *form* of the cells.)

(3) No general law can be deduced as to the equality or inequality of the divisions.

(4) There is a tendency for the largest cells to cleave fastest. Certain very small cells (at the dorsal pole) do not cleave at all.

Other Changes during the Seventh Cleavage. — In almost every cleavage which has taken place, whenever the division was equatorial, — the spindles taking a dorso-ventral position, — it will have been noticed that the axis in which the spindle was formed was the short axis of the cell. On the other hand, the cells in which meridional cleavages have taken place have commonly been already somewhat elongated in the direction of the spindle. Therefore, at the occurrence of division, the short cells, cleaving equatorially, have changed form greatly, becoming more elongated dorso-ventrally, while the cells which cleave meridionally have already been of sufficient length to permit of the extension of the spindle without much change of form. As a result, there has been a great extension of the ectoderm dorso-ventrally. This produces first a complete closure of the blastopore. (Compare Figure 65, Plate **8**, with Figure 76, Plate **9**.) A second result, due to the larger size of the cells of the quadrant *D*, and perhaps partly also to the fact that they cleave first, is the further displacement of the animal pole of the egg from the micromere end toward the anterior side (Plate **8**, Fig. 65, Plate **9**, Fig. 76).

The closure of the blastopore is not sufficient to provide for the dorso-ventral extension brought about during the cleavage, so that, as a *third* consequence, the cells at the ventral pole of the egg, where the blastopore was previously situated, are pushed over or under one another, the ectoderm tending to become two-layered in this region. A comparison of Figure 63 (Plate **8**) with Figure 73 (Plate **9**) shows the resulting conditions. In the latter figure the entoderm ($d^{8.1}$ in Fig. 63) is entirely enclosed, and the ventral cells $a^{7.1}-c^{7.1}$, $a^{7.2}-c^{7.2}$, $a^{7.3}-c^{7.3}$, and $a^{7.4}-c^{7.4}$ have become crushed together, and several of them, as $a^{7.2}$ and $b^{7.3}$, are almost hidden by surrounding cells. The beginning of the two-layer condition is shown in frontal section in Figure 80 (Plate **10**), from the same egg as that shown in Figure 73 (Plate **9**).

Meanwhile other cleavages are taking place in quadrant *D*, leading to still further modifications of the structure of the egg. It is therefore necessary to return to a consideration of this quadrant.

Quadrant D. — By reference to the diagram of the cells of this quadrant at the end of the seventh cleavage (Diagram IV., page 48), it will be seen that there are now present, exclusive of the entoderm, twenty-three cells, arranged somewhat irregularly. Approximately the same stage, as it actually appears, is shown in Figure 68 (Plate **8**).

In this egg spindles have appeared in the large cells $d^{8.11}$ and $d^{8.12}$, and later they each become divided into two equal cells. It is worthy of notice that the two spindles are not parallel, but make a slight angle with each other. The two cells lie on opposite sides of the median dorso-ventral plane, so that the angle between the spindles indicates the beginning of a tendency to *bilateral* cleavage. The inclined position of the other spindles (in $c^{7.7}$ and $c^{7.11}$) is indicative of the same fact. The tendency toward bilaterality is apparently due to the crowding of the cells from all directions toward the blastoporic region, which lies at the ventral border of $d^{8.9}$ (Fig. 68). The cells resulting from the division of $d^{8.11}$ and $d^{8.12}$ are $d^{9.21}$, $d^{9.22}$, $d^{9.23}$, and $d^{9.24}$; they are shown in Figure 74 (Plate **9**).

Next the four cells immediately dorsad of these, $d^{8.17}$, $d^{8.18}$, $d^{8.19}$, and $d^{8.20}$, develop spindles in their short axes (Plate **8**, Fig. 68), which as a result of extension become the long axes (Fig. 74, Plate **9**), — the cleavage being in each case equatorial and equal.

Now the four large cells $d^{8.25}$, $d^{8.26}$, $d^{8.27}$ and $d^{8.28}$, shown in the dorsal part of Figure 68 (Plate **8**), cleave equatorially also.

As a result of these many equatorial cleavages the quadrant *D* becomes greatly increased in dorso-ventral extent. The animal pole is forced farther upon the anterior side of the egg, toward the blastopore, so that the cells of the quadrant *D* come to occupy in the region of a sagittal section much more than half the circumference of the egg (Plate **9**, Fig. 76).

The two ventral cells $d^{8.9}$ and $d^{8.10}$ meanwhile divide meridionally, completing the separation of quadrant *D* into two portions lying on each side of the median line (Fig. 74). However, the egg is not yet completely separated by cleavage planes into right and left halves, for the entodermic cell $d^{9.4}$ occupies the median plane at even a later stage than this (Plate **10**, Fig. 83).

This is the latest stage to which I have traced the cleavage in the ectodermal part of quadrant *D*. Diagram VI. shows the condition at this time.

The dorso-ventral extension of the ectoderm, and consequent crowding together of the cells in the region of the blastopore, are still further increased by the eighth cleavage of the large cells $a^{8.25}-c^{8.25}$, $a^{8.26}-c^{8.26}$, $a^{8.27}-c^{8.27}$, and $a^{8.28}-c^{8.28}$, belonging to the other quadrants, which likewise divide equatorially and equally (Plate **9**, Fig. 75).

The blastoporic region has now become distinctly two-layered, as shown in Figure 77. The cells of quadrants *A*, *B*, and *C* are turned in and pushed dorsad, in the same manner as happened in early stages to the large ventral cell of quadrant *D*. The anterior lip of the blastopore thus becomes two layers thick, while the posterior lip is formed of a single layer of cells from the quadrant *D*, resting against the entoderm cells. Between these ventral cells of quadrant *D* and the infolding cells of the other quadrants, a slight notch appears, marking the position of the blastopore. (At an earlier stage the blastopore was entirely closed, as shown in Figure 73.) The blastopore notch lies, not directly at the macromere end of the egg, but at some distance on the posterior side of it.

As the cells of the anterior lip of the blastopore are turned inward, some of them begin to divide. Spindles in these cells are shown in Plate **9**, Figs. 75 and 77. The form and position of the cells have changed so much at this time that it is impossible to determine with certainty whether the cleavage should be considered equatorial or meridional. In the cases figured the spindles are nearly or quite transverse, so that in some of the cells the division is meridional.

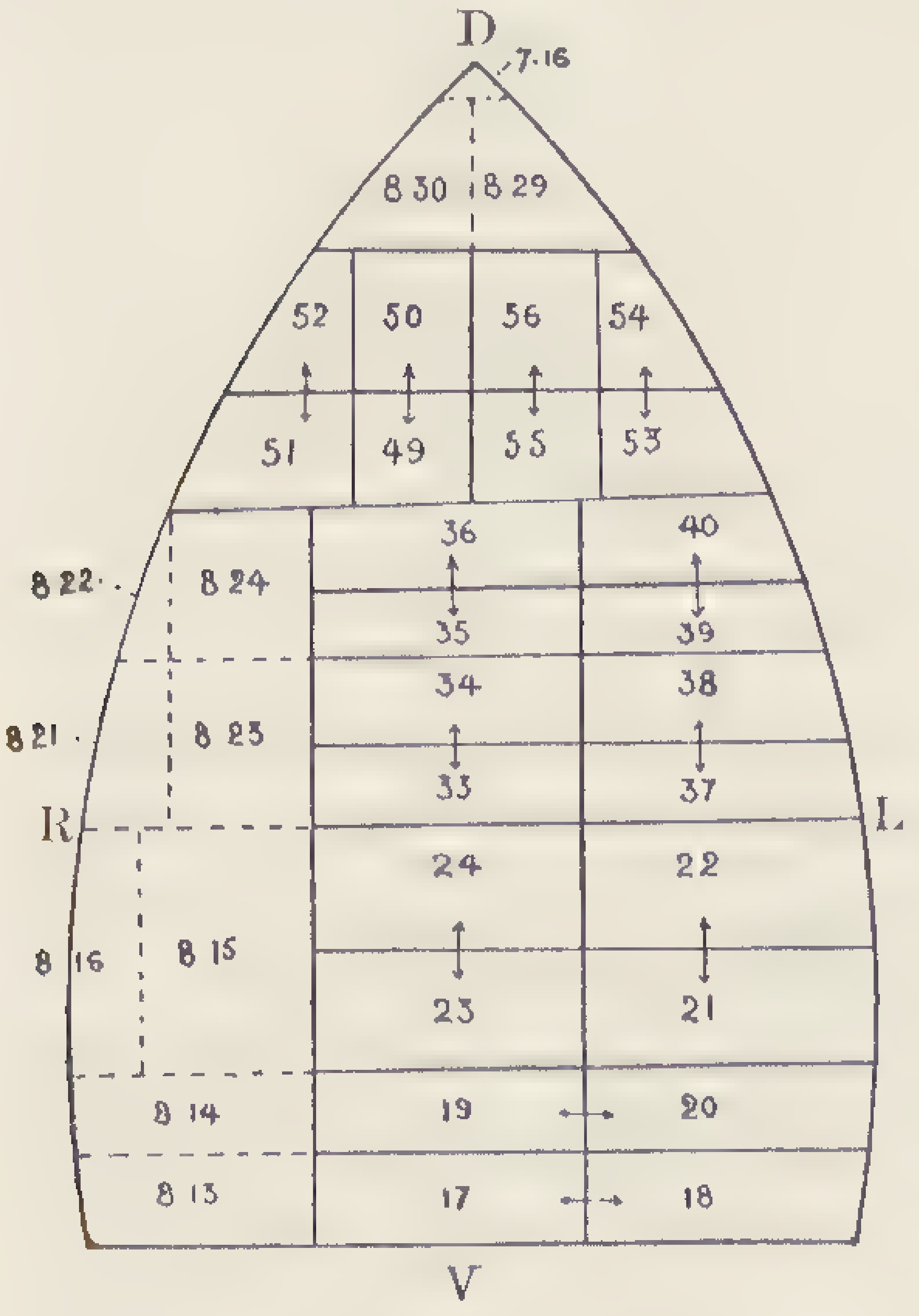

Diagram VI.

Diagram of quadrant *D* at a time when most of the cells have passed into the ninth generation. All cells in the ninth generation are bounded by continuous lines, and are designated by the second exponent belonging to the cell, the first exponent being in each case 9. In the other cells both exponents are given. The arrows connect cells of common origin, and show the direction of the spindle at the last division.

R signifies right; L, left; D, dorsal; V, ventral.

The cleavage of the ectoderm has now been traced to the eighth generation in all parts of the egg, and in the greater part of the quadrant *D* to the ninth generation.

With this ends the account of the cleavage of the ectodermal cells. The small size and the great displacements of the blastomeres, especially in the regions of the blastopore and the animal pole, render it impossible to determine with certainty their identity in later stages, and the real direction of cleavage is masked by crowding and deformation of the cells. It would perhaps be of little interest in connection with the laws of cleavage to carry the study further, as it is scarcely to be presumed that the later divisions would exhibit any phenomena differing fundamentally in kind from those already shown in the earlier stages.

We must now turn to a consideration of

The Entoderm.

The cells which I have called the entoderm — following in this Zelinka ('91) and Tessin ('86) — are those derived from the single large cell of the quadrant *D*, which passes within the egg in the manner already described. I have already given an account of the first cleavages of this cell after it has become partially covered by the other blastomeres. As will be recalled, at the fifth and sixth cleavages the spindles occupied twice in succession the same position, one end lying in the anterior median line, between the ventral cells of quadrants *A* and *B* (Plate **5**, Fig. 35, and Plate **6**, Fig. 48). Here was given off at each of these cleavages a minute vesicle, the entire process being comparable in external features to the successive formation of two polar cells at a given spot on the surface of the egg. The two vesicles thus formed maintain their position for some time (Plate **7**, Fig. 55), but as the surrounding cells become invaginated, I have found it impossible to follow their later fate.

We will follow the cleavage of the large cell $d^{7.1}$ (Plate **6**, Fig. 50), which forms the greater bulk of the entoderm.

After the sixth cleavage (Fig. 49), the asters in $d^{7.1}$ at once separate nearly in the dorso-ventral axis of the egg, as shown in Figure 50. The line joining them is at first a little oblique, the ventral aster being a little to the right. This obliquity soon corrects itself, and the asters come to lie in the sagittal plane. As the spindle is formed, its dorsal end moves to the posterior side, so that the spindle is no longer in the dorso-ventral axis of the egg. This stage is shown in Figure 64 (Plate **8**); as may be observed in this figure, the spindle is neither in the longer axis of the cell nor at right angles to it, but oblique. The cloud of granules, which soon after the last division occupied a region on the anterior side of the cell, underneath the two vesicles $d^{6.2}$ and $d^{7.2}$, now surrounds the aster at the dorso-posterior end of the spindle.

The cleavage is unequal, separating (Fig. 65) a smaller dorso-posterior cell, $d^{8.2}$, from a larger anterior one, $d^{8.1}$. The cloud of granules remains in the smaller, dorsal cell, forming a band about its periphery, so as to leave a free space surrounding the nucleus. The position of the animal pole of the egg with reference to this cell should be carefully noted, as the relation remains constant, at least for a time, during the considerable shiftings which take place. As seen in Figure 65, the animal pole lies at the anterior margin of the cell $d^{8.2}$.

Before the *eighth cleavage* takes place, the blastopore has become closed and its anterior margin has begun to pass into the two-layer condition, as previously described. The larger cell, $d^{8.1}$, divides before its mate, by a spindle at right angles to the previous spindle. The cleavage is equal, forming the two large right and left cells $d^{9.1}$ and $d^{9.2}$, shown in Plate **10**, Fig. 80. The plane separating these cells coincides with that separating the quadrants A and B on the anterior side of the egg, and also with that separating the two cells $d^{8.11}$ and $d^{8.12}$ on the posterior side (Plate **8**, Fig. 68). As this plane also passes through the animal pole and the blastopore, it divides the egg into symmetrical halves, and is the median dorso-ventral or sagittal plane of the embryo.

Later the small cell, $d^{8.2}$, develops a spindle in the same direction as the spindle of the seventh cleavage, in the shorter axis of the cell, and divides into two very unequal cells. The anterior or ventral cell, $d^{9.3}$, is a minute vesicle, whereas the dorsal or posterior blastomere, $d^{9.4}$, is scarcely smaller than the mother cell. The process of budding off this small cell is shown in Plate **10**, Figure 80. The vesicle lies between the two large cells $d^{9.1}$ and $d^{9.2}$, and, like the minute cells $d^{6.2}$ and $d^{7.2}$, can be traced but a short distance, soon becoming lost among the many cells by which it is surrounded.

In the *ninth cleavage*, spindles are formed in the cells $d^{9.1}$ and $d^{9.2}$ in the position foreshadowed by the asters in Figure 80, — that is, antero-posterior, and at right angles to the preceding spindles, — and the cells divide equally, forming the four cells $d^{10.1}$, $d^{10.2}$, $d^{10.3}$. and $d^{10.4}$. Figures 76 and 81 show the entoderm at the close of these divisions — the nuclei of the cells in question being still connected in pairs by interzonal filaments. The blastopore is now present as a distinct notch; its anterior or dorsal lip has become two-layered, owing to the folding inward of the cells of the anterior quadrants. The animal pole (*pol. anm.*, Fig. 76) has moved a considerable distance on to the previously anterior surface, lying still at the anterior margin of the entoderm cell $d^{9.4}$. As the side view (Fig. 76) shows, a frontal plane carried through the long axis of the egg at this stage would cut the nuclei of all the entoderm cells, as realized in the frontal section, Figure 81 (Plate **10**).

As soon as the cells, after the cleavage process is entirely finished, have lost their strong tendency to maintain a form as nearly spherical as possible, a sudden and considerable change of relative position takes place. The four entodermal cells, $d^{10.1}$–$d^{10.4}$ become much flattened dorso-ventrally, and the invagination of cells at the anterior lip of the blastopore increases in extent. These cells press most strongly upon the anterior surface of the ventral entodermal cells, forcing them toward the posterior side. An early stage in the process is shown in Plate **9**, Figure 77. Two of the cells of the invaginating ectoderm have flattened themselves against the entoderm cells in such a way as to form a direct continuation of the longitudinal series of interior cells. This longitudinal series is however tending to become curved by the displacement of the cell $d^{9.4}$ in a dorsal and anterior direction. This cell, together with the animal pole, has moved a very slight distance toward the macromere end of the egg. The animal pole now lies (Fig. 78) directly above the plane separating the cells $d^{10.1}$ and $d^{10.3}$ from $d^{10.2}$ and $d^{10.4}$, instead of above the posterior margin of the cells $d^{10.2}$ and $d^{10.4}$, as previously. A frontal plane carried through the long axis of the egg would not now cut the cell $d^{9.4}$ at all. Figure 82 shows a view from the animal pole, the outer layer of ectoderm cells being supposed to be removed from the dorsal half of the egg, while the entoderm cells remain in position.

This process of *rotation* of the entodermic contents, as one might call the phenomenon, continues still farther. Figure 79 shows a stage in which the process is much more advanced. The ectodermal plug at the anterior lip of the blastopore has become very much thickened, and projects farther posteriad; the four large entoderm cells $d^{10.1}$–$d^{10.4}$, of which of course but one pair is shown in the side view, are now so displaced that the plane separating the pair, which previously lay in the short axis of the egg (Figs. 76 and 78), now lies in the long axis (Fig. 79). The line connecting the centres of a given lateral pair is now at right angles to the line previously connecting them.

At this time the five large cells constituting the entoderm — the minute cell $d^{9.3}$ not being traceable farther — begin to undergo karyokinetic changes preparatory to division. The spindles in various stages are seen in three of the cells in the side view, Figure 79; the same stage, slightly earlier, is shown from the animal pole in Figure 83 (Plate **10**), in which the covering ectoderm is supposed to have been removed from the dorsal side of the egg. As a comparison of Figures 79 and 83 shows, the spindles do not lie in parallel planes, so that no single view can give a complete representation of their positions. Nevertheless, Figure 83 shows that the arrangement is distinctly bilateral. The cell $d^{9.4}$ lies in the middle line, with its spindle in the sagittal plane of the egg; the spindles in the other cells all radiate outward from the region occupied by $d^{9.4}$, the sagittal plane passing through this cell forming a plane of symmetry for all. The median plane thus indicated coincides with that already defined by the line separating the quadrants *A* and *B* anteriorly, and the boundary between the cells $d^{8.11}$ and $d^{8.12}$ posteriorly; it passes through the animal pole and the blastopore.

This movement of the entodermal blastomeres is of course simply a continuation of the rotation inaugurated at the passage from the four- to the eight-cell stage. The ectodermic cells continually withdraw their deeper parts and increase their surface area at division; in this way cells are continually forced in at the blastopore. These press upon the anterior and ventral aspects of the entoderm cells, forcing them backward, as already described. The pressure is greatest in the median region, so that the anterior or ventral ends of the cells on the two sides of the median plane are forced apart, the axes of the cells become oblique to the long axis of the embryo, and the oblique position of spindles shown in Figure 83 results.

Beyond this point it is impossible to trace the development cell by cell. In Asplanchna there is a period, intervening between the stage to which it is possible to trace the cleavage step by step (about 120 cells) and the stage of recognizable differentiation of organs, during which the cells divide and become very minute. The cells probably reach the number of from 250 to 500, and the process of extension of cells and consequent "rotation" and invagination of parts of the embryo continues. A sagittal section of the embryo at about the time of the beginning of differentiation of organs is shown in Figure 84. It thus becomes impossible to trace the fate of individual blastomeres, or even, except in a most general way, the fate of the different regions of the embryo during the later portion of the cleaving. From the small size of the adult rotifer, I had hoped that it would prove to be a favorable object for an exact study of the cytogenetic history of organs; in Asplanchna this turns out not to be the case. But, on the other hand, for a study of the factors in the early developmental processes it has shown itself well fitted.

My study of the processes in the early development of Asplanchna therefore closes with the stages shown in Figures 79 and 83 (Plates **9** and **10**).

For critical comparison of my observations with those of Zelinka and other workers on the development of the Rotifera, the reader is referred to Part Second.

I shall now proceed to a discussion of the bearing of the foregoing observations upon the problems already proposed, as well as upon other related subjects.

III. Discussion of the Bearing of the Observations on the Problems.

In the following discussion I shall adopt in general the order pursued in my "Statement of Problems," taking up successively the various theories in regard to cleavage and gastrulation, and pointing out what bearing the observations above detailed have upon these theories. This will be followed by a *résumé* of the general conclusions which may be drawn from the work.

We will therefore first take up a discussion of the cleavage, and of the theories bearing upon it.

1. Cleavage.

A. The Direction of Cleavage.

(1) *Berthold's theory of surfaces of least area.* (See page 4.) — The two- and four-cell stages in Asplanchna agree well with the conditions demanded by the law of least surfaces. The peculiar arrangement of blastomeres in the four-cell stage, agreeing as it does with the four-cell stage of animals of the most various systematic positions, and with the four-cell stage of many plants, seems probably due to some very general law. In all these cases only three cells meet along one common line. As this is the arrangement demanded by the principle of least surfaces, the conclusion seems perhaps justifiable that this principle of least surfaces is that common law.

The eight-cell stage also fulfils the requirements of the principle of least surfaces. But from this stage onward, many of the conditions found are irreconcilable with the view that this principle is a determining factor. Six of the cells in the eight-cell stage divide in a manner that squarely defies the principle of least surfaces. Nor does the arrangement of cells in the resting periods agree better with the principle. As pointed out on page 30, the flat, almost disk-shaped form taken by the cells of quadrant *D* during the ten-cell stage (Figs. 23 and 25, Plate **3**) and the sixteen-cell stage (Figs. 31 and 32, Plate **4**) is widely at variance with the demands of the principle of least surfaces. The form of the cells in quadrants *A*, *B*, and *C* during their resting period in the sixteen-cell stage (Figs. 30 and 34) is equally impossible of explanation on the least surfaces theory. Many other cases could be adduced in which this principle is contradicted, but a fuller discussion of these cases will be given under the next theory (Hertwig's law). In general, any case which is not in agreement with Hertwig's law is likewise inexplicable by Berthold's principle, so that all the cases cited later as opposed to Hertwig's law can be utilized equally well against the principle of least surfaces as a determining force.

It should be noted that Berthold did not in any sense maintain that this principle is the *decisive* factor in cell division, or the arrangement of cells in tissues. He recognized that the conditions in a living cellular body are widely different from those in a simple vesiculated fluid, and that the conditions actually found in plant tissues are often inexplicable by the principle of least surfaces, — in many cases, indeed, directly opposed to it. "Aber nothwendig ist das in der Zelle nicht, wie bei den Flüssigkeitslamellen. Denn wir sahen schon früher, dass die Symmetrieverhältnisse der Zellen von der äusseren Form oft vollständig unabhängig werden, und auch unter Mitwirkung der äusseren Formverhältnisse können bei dem ineinandergreifen der verschiedenen Factoren sich Theilungsrichtungen ergeben, die mit den Forderungen des Princips der kleinsten Flächen nicht in Uebereinstimmung stehen." (Berthold, '86, p. 230.) Berthold ('86, p. 230, Taf. 4) describes and figures many cases in which the arrangement and division of cells is not in accordance with the principle of least surfaces.

The fact that a single cell may at one time take such a form as that which $d^{6.3}$ shows in the surface view in Figure 37 (Plate **5**), and in section in Figure 38, and at another time have the form exhibited by the same cell in Plate **7**, Figure 54 (surface), and Plate **6**, Figure 48 (section), while the shape of the egg remains unchanged, demonstrates that we are not here dealing with a problem of the statics of a vesiculated fluid; a single simple principle can no more account for the forms taken than it can for the protean changes of shape of an Amœba.

By this I of course do not mean to imply that it is not possible, and perhaps probable, that the laws of surface tension do, within certain limits, modify the form and arrangement of cells, as maintained and discussed at length by Berthold. Wherever the arrangement demanded by the principle of least surfaces is not in conflict with other purposes of the organism, or, to put it upon a less teleological basis, where it is not in conflict with stronger influences than the force of surface tension, the cells probably accommodate themselves to the demands of that principle. The point of importance, however, is that this is not a *decisive* factor, but may at once be overcome when other influences in the organism antagonize it.

Zimmerman ('93) holds that the general arrangement of cells in accordance with the principle of least surfaces in plant tissues is not due to surface tension at all, but to turgor. Turgor, however, can hardly be a factor in the cleavage of the egg, where no increase in size is taking place.

(2) *Hertwig's law of the spindle in the longest axis of the protoplasmic mass.* (Compare page 5.) — This is, so far as I know, the only principle for which the claim is made, that it is *the* decisive factor in determining the direction of the spindle. The statement quoted above (page 5) is from Hertwig's general text-book on the subject, in which it is presumable that care would be taken not to mislead the reader unacquainted with the literature into taking a special phenomenon for a law of general import. On the same page it is stated that "Mit diesen Regeln stimmen die Erscheinungen, wie sie bei der Zelltheilung und insbesondere bei der Eifurchung beobachtet werden, fast ausnahmslos überein." (Hertwig, '93, p. 175.) The "law" has been accepted by others in the same general bearing. Thus Ziegler ('94, p. 154) questions the validity of cases apparently not in agreement with the rule, holding that they are due either to inexact study (cylindrical epithelium), or to the difficulties of determining in the presence of a mass of yolk (amphibian egg) which *is* the longest dimension of the protoplasmic mass. This principle has become the most widely known and generally accepted of any of the principles which have been proposed in regard to the determining factors in cell division. I shall therefore discuss it at somewhat greater length than Berthold's principle, analyzing in detail the evidence on the subject from my own work, and reviewing that advanced by others.

A comparison of the very first cleavage of Asplanchna with that of Callidina (Zelinka, '91) shows that in the Rotifera the form of the egg is not the factor determining the position of the first cleavage spindle. For in the two forms the first cleavage spindle bears the *same* relation to the animal pole, or place of polar-cell formation, but a *different* relation to the long axis of the egg. In Asplanchna the spindle at the time of division lies in the long axis of the egg (though a little earlier it is oblique), whereas in Callidina (Zelinka, '91, Taf. I. Fig. 5) the spindle at division is oblique to both the longer and shorter axes, — the place of polar-cell formation not being the same as in Asplanchna, but much nearer one end of the egg. The orientation of the spindle in these two rotifers, then, is constant with reference to the animal pole, but variable with reference to the form of the egg.

The passage from the eight- to the sixteen-cell stage in Asplanchna is particularly instructive. The asters of the six cells of quadrants *A*, *B*, and *C* first separate in such a way that the line joining them lies in the long axis of the cell (Plate **3**, Fig. 17), then a rotation takes place (Fig. 18) by which the line joining the asters, i. e. the axis of the future spindle, is brought into the shortest axis of the cell (Figs. 19–24). The six spindles are then formed in the shortest axes of the cells (Figs. 20–28), and the *planes* of cleavage accordingly coincide with the long axes of the cells.

The simple fact that there are divisions in which the spindles lie in the shortest axis of the cells is of course a direct contradiction of Hertwig's law. The case becomes even more striking, however, when the movements of the asters are taken into consideration. They *at first* lie in the position demanded by the law, but *move from* this position to that which directly contradicts the law. (See pages 25, 26.)

Hertwig ('93, p. 175) has cited a similar phenomenon, described by Auerbach, as proof of his law. Auerbach ('74) observed in the eggs of Ascaris nigrovenosa, at the time of fertilization, that the two pronuclei often come together in such a way that the plane separating them lies in the short axis of the egg. Since the axis of the first cleavage spindle commonly coincides with the plane separating the pronuclei, the result in the eggs of this species of Ascaris would be that the spindle would occupy the short axis of the egg. But the two pronuclei after meeting undergo a rotation through an angle of 90 degrees, thus bringing the spindle into the *long* axis of the egg. Ziegler has recently observed with even greater clearness the same phenomenon in the eggs and cleavage cells of other nematodes (Ziegler, '95, Taf. XVIII. Figs. 40–42), and in the eggs of echinoderms ('94). He observed in nematodes in some cases that the line joining the two asters on opposite sides of the nucleus lies in the short axis of the egg, and that then follows a rotation of the whole complex, till the line joining the asters — the axis of the forming spindle — occupies the longest axis of the egg. Ziegler, like Hertwig, has interpreted this change of position as a confirmation of Hertwig's law, and the interpretation is certainly the most natural and apparently well grounded that could be given.

Nevertheless we have in Asplanchna an entirely similar phenomenon, but occurring under such circumstances as to give a direct contradiction, instead of a confirmation, of Hertwig's law.

It is instructive also to notice that in the eight-cell stage of Asplanchna, notwithstanding the great variety in the form of the cells, the direction of the cleavage spindles is the same in all the cells. Thus $d^{4.1}$ (Plate **2**, Fig. 15), though irregular in shape, is of such a form that it is possible to be confident that the spindle does lie in the greatest dimension of the cell. The blastomere $d^{4.2}$ (Plate **2**, Figs. 14 and 16) is so exceedingly irregular in form, that it is impossible to determine with certainty which is the longest axis. The cells $a^{4.2}-c^{4.2}$ are irregularly triangular, and the direction in which the spindle lies is the shortest line connecting any apex with the middle of the opposite side (Plate **3**, Figs. 18, 19). Finally, the cells $a^{4.1}-c^{4.1}$ are approximately rectangular in form, with one of the axes much longer than the other; the spindles lie in the shorter axes (Figs. 20–22). In every case the spindle, whatever the form of the cell, lies in a meridian connecting the animal pole with the vegetative pole of the egg. The only rational conclusion from this fact is, that the position of the spindles is determined by some factor unconnected with the form of the cells.

The theory that the direction of the spindles is due to their taking a position of *equilibrium* determined by the mutual attraction of spindle and protoplasm, so strongly insisted upon by Ziegler ('94, p. 140), is likewise inconsistent with the movements of the asters in the eight-cell stage of Asplanchna. Ziegler holds that, since the greater mass of protoplasm must exercise the greater attraction, the spindle in the short axis of the cell is in a position of unstable equilibrium; if by any cause it is moved in the slightest degree to one side or the other, it must inevitably swing into the long axis of the cell, where alone it can be in a position of stable equilibrium. This he holds to be the explanation of the movements of asters and nuclei observed by Auerbach in nematodes, and by himself in nematodes and echinoderms, as mentioned above. An oblique position of asters and nuclei, such as is shown in the cell $a^{4.1}$, Figure 22 (Plate **3**), is intelligible on this assumption if the movement taking place is from the shorter toward the longer axis. But in this cell, as in the other five of the quadrants A, B, and C at this stage, the movement is from the longer axis toward the shorter. The hypothesis that the movement is due to simple attraction between the protoplasm and the fundament of the spindle, varying with the *mass* of the protoplasm, is totally inconsistent with such a notion.

The passage from eight to sixteen cells is not the only cleavage in Asplanchna which is irreconcilable with Hertwig's principle. At the transition from the sixteen-cell to the thirty-two-cell stage, there is a similar regularity in the position of the spindles coincident with variety in the form of the cells. The four dorsal cells $a^{5.4}-d^{5.4}$ divide equatorially, three of them with spindles in the longer axis; one, $d^{5.4}$, with the spindle in the shorter axis (Plate **4**, Fig. 33).

Again, in the sixth cleavage the cell $d^{6.3}$ shows the same phenomenon of rotation of the spindle into the shorter axis of the cell (Plate **5**, Fig. 37, Plate **6**, Fig. 46, and Plate **7**, Figs. 53 and 54). There is the same regularity in the direction of cleavage as noticed in the preceding cleavages, although there is variation in the form of the cells. Thus, $d^{6.4}$, the companion cell of $d^{6.3}$, whose division with spindle in the short axis has just been cited, cleaves with the spindle parallel with that in $d^{6.3}$, but owing to the form of the cell the spindle in $d^{6.4}$ occupies the long axis (Plate **7**, Fig. 57, cleavage finished). The cells of the ventral layer (Plate **6**, Fig. 47) divide with spindles in the same direction as those in the cells of the second layer, although in the one layer the spindles must thereby occupy the short axes of the cells, in the other the longer axes.

In the seventh cleavage a still more striking case occurs. The middle of the embryo is surrounded by two rows of eight cells each, of precisely the same form and size, the dorsal row composed of the blastomeres $a^{7.6}-d^{7.6}$ and $a^{7.8}-d^{7.8}$, the ventral row of $a^{7.5}-d^{7.5}$ and $a^{7.7}-d^{7.7}$. These two rows are shown in Figure 61 (Plate **7**), for the quadrants *A*, *B*, and *C*, and in Figure 57 for the quadrant *D*. In both rows, every cell but one in each row ($d^{7.7}$ and $d^{7.8}$, Fig. 57) is strongly flattened dorso-ventrally, so that the lateral extent of the cell is much greater than the dorso-ventral extent. If the form of the cell determines in any way the direction of the spindle, it is certainly to be anticipated that the direction of cleavage will be the same for the cells of both rows. On the contrary, *all the cells of the dorsal row divide meridionally*, while *all the cells of the ventral row divide equatorially*. In every cell of the dorsal row, except possibly $d^{7.8}$, the spindle lies in the *long* axis of the cell; in every cell of the ventral row, except $d^{7.7}$, the spindle lies in the *short* axis of the cell. The exception of a single cell in each row gives a finishing touch to the proof that the form of the cells is not the factor determining the direction of cleavage. The fact that the cell $d^{7.8}$ divides with its spindle in the same direction as the spindles of all the cells in the same row, but not, as in the other cells, in the long axis of the cell, while $d^{7.7}$ likewise divides with its spindle in the same direction as those of the other cells of *its* row, but not, like them, in the short axis, demonstrates that the dorsal row is not so constituted that all the spindles must take their positions in the long axis, nor the ventral row so that all must take their positions in the short axis. It demonstrates, in other words, that the relative dimensions of the different axes of the cells does not determine the direction of the spindles in either one way or the other. (The cleavage of these two series of cells is shown for the quadrants *A*, *B*, and *C* in Plate **8**, Figs. 69 and 70; in $d^{7.6}$ in Plate **7**, Fig. 58; in $d^{7.5}$ in Plate **8**, Fig. 66; in $d^{7.7}$ and $d^{7.8}$, in Fig. 67.)

Still other divisions in which the spindle lies in the short axis have been followed out in the descriptive portion of this paper.

We must conclude, therefore, that a very large number of cell divisions in the cleavage of Asplanchna directly contradict Hertwig's law that the spindle during division comes to lie in such a position that its axis coincides with the greatest axis of the protoplasmic mass. The characteristic feature of the cleavage is *regularity* in the direction of the spindles, coupled with great *variation* in the form of the cells, thus excluding any close relation between these phenomena.

What is the evidence upon which this law has been based?

It is chiefly experimental, though there is likewise a certain amount of evidence based upon the observation of normal cleavage.

Let us consider first the evidence derived from experiment. The experimental studies of Pflüger ('84), Roux ('85), Driesch ('92), Hertwig ('93ª), Born ('93 and '94), Ryder ('93), and Ziegler ('94), on the effects of pressure upon the direction of the spindle, are so well known that it is not necessary to review them in detail. It is sufficient to state the general result. With rare exceptions it has been found that when the egg or the cleavage cell is so modified in form that one of the axes which may be passed through its protoplasmic mass is distinctly greater than the others, the spindle at cleavage comes to lie in this axis. I do not propose to enter upon an analysis of these experiments, nor to attempt to explain in any different manner the results gained. A study of the works of the authors above cited, and a repetition of the pressure experiments upon the eggs of the toad (Bufo lentiginosus Shaw) during the spring of 1895, have convinced me that the explanation commonly given is the one most in agreement with the conditions, and, from the evidence, most probably correct, *for these cases*. But whatever we may hold as to the validity of the explanation for these cases, we know that the principle upon which it is based cannot be *generalized*, since in many other cases it is directly contradicted by the facts. Before suggesting how the experimental results may perhaps be reconciled with the apparently contradictory phenomena observed in other cases, it will be necessary to consider the evidence gained by other means, as well as such experimental evidence as is *against* the principle.

First, then, we have the fact that the experimental evidence itself is not concordant upon this point. Roux ('85) found that under certain circumstances in deformed frogs' eggs the first cleavage plane sometimes, though rarely (Roux, '94^a, p. 274), passed through the greater axis of the cell, the spindle therefore lying in the shorter axis. Eycleshymer ('95) experimented as to the effects of pressure on the eggs of Amblystoma tigrinum, and found that when the eggs were compressed laterally to *one half* their normal equatorial diameter, there was little or no relation between the direction of cleavage planes and the greater or less dimensions of the egg. "The first vertical in the thirty-four eggs examined showed no constant relation to the compressed surfaces, in seven passing through the longest equatorial diameter; in nine through the shortest; in eighteen between the two." (Eycleshymer, '95, p. 353.) In experiments of a different nature, Morgan ('95) observed that the shaken eggs of Sphærechinus often divide at the first cleavage into three equal blastomeres, and in such cases at the *next* cleavage the three spindles lie in the short axes of the cells.

When we turn to the evidence from observation of normal cell division, there is the same disagreement that is met with in the experimental evidence. Until within a few years investigators in zoölogical lines have not paid attention to the exact relations of the spindle to the cell axes, so that little was to be found in the literature to emphasize the necessity of caution in accepting at once the generalizations from the first experimental results. In cases where cells containing spindles were figured, there was generally an apparent agreement with the principle that the spindles are in the long axis, but so long as it was not determined by observation whether this elongation of the cell was a *consequence* of the position of the spindle or a *cause* of it, the evidence was worthless, as pointed out by Heidenhain ('94), and as clearly illustrated in the preceding pages.

In botanical literature the case was somewhat different, and seven years before Hertwig ('93) had stated that the phenomena observed in cell division agreed "fast ausnahmslos" with his law, Berthold ('86, p. 230), in his thorough and comprehensive work on the subject, had said: "Sehr oft theilen sich prismatische oder cylindrische Zellen der Länge nach, wenn das Prinzip [of least surfaces] eine Querwand, der Quere nach, wenn es eine Längswand verlangte. So theilen sich oft die Markzellen, die Zellen der Grundparenchyms sich entwickelnder Blätter nur quer, *obwohl ihre Höhe im Vergleich zur Breite nur gering ist*" [Italics mine]. He had also given many examples of the conditions thus characterized.

Recently the attention of zoölogists has been directed to a careful observation of these relations, and a number of facts are now at hand which bear upon the subject.

Ziegler ('95) has studied the cleavage of certain nematodes (Diplogaster longicauda, Rhabdites teres, and Rhabditis nigrovenosa) with especial reference to the relations of cell form to the direction of cleavage, and finds that the conditions in these cases *confirm throughout the law of Hertwig.* In the normal cleavage the spindle always places itself in the long axis of the cell, even though a rotation of the nucleus and asters from their first position is often necessary to accomplish this result. And in cases where the egg is deformed by some outer agent, as pressure by the walls of the oviduct or the like, the normal position of the spindle is changed to agree with the changed form of the cells, the spindle lying in the long axis in every case. The agreement with Hertwig's law is complete.

On the other hand, zur Strassen ('95 and '96) has studied the cleavage of another nematode, Ascaris megalocephala, with the same questions in mind, and has come to *opposite* results. In the two-cell stage one cell divides with the spindle in the long axis, the other with the spindle in the short axis, and in later stages a similar independence of the position of the spindle and relative dimensions of the cell is shown. Zur Strassen ('95, p. 86) concludes: "Ich halte vielmehr den Kern für befähigt, vermöge ihm inhaerenter Eigenschaften eine gewollte Theilungsrichtung herbeizuführen, selbst wenn mechanische Hindernisse von nicht unbedeutender Höhe dem entgegenstehen."

Other observations bearing upon this question are much scattered. Cases are not uncommon in which authors have figured spindles in the shorter axes of the cells, but unless the observer's attention has been especially directed to the question, such figures are of little value, since a slight change in the position from which the cell is viewed produces a foreshortening which gives very different apparent relative dimensions to the axes. Heidenhain ('95, p. 553) gives a number of such cases, from most of which the evidence is weakened by this consideration. However, the case mentioned by Heidenhain of the germinal disk of the cephalopod egg as figured by Watase ('91) cannot be explained away upon this ground.

Some other instances may be mentioned.

In the formation of the "germ bands" in the Polychæt Amphitrite, according to Mead ('94, p. 467), "the axes of the spindles in these divisions lie in the shortest diameter of the cells, and apparently in the direction of greatest pressure."

Wheeler ('95, p. 309) states in regard to the first cleavage spindle of Myzostoma: "In Myzostoma the spindle does not conform to O. Hertwig's law, but always lies at right angles to the long axis of the often very narrow protoplasmic pillar of the egg."

Castle ('96, p. 250) states that in the division of the entoderm cells of Ciona the spindles in certain cases lie in the short axes of the cells, even when a shifting of the asters from a previous position in the long axis must have occurred to bring about this condition. Castle states that no mechanical explanation of this phenomenon offers itself, though he holds that "*other things being equal*, it is true that the spindle arises in the longest axis of the cell" (p. 231, note).

In the decapod crustacean Virbius, according to Gorham ('95), the egg is ellipsoidal in form, and the first cleavage spindle may occupy the long axis, or be more or less inclined to it, or may even be nearly at right angles to it.

The cell divisions in the germ bands of Crustacea as described by Bergh ('95), in which the spindles take the same direction for many cell generations, should be added here (see also McMurrich, '95); though the evidence from these must be weakened in the eyes of the upholders of Hertwig's law by the fact that before division the cells "wachsen nur in der Weise, das ihre Längsdurchmesser dem Querdurchmesser ziemlich gleich wird und dann tritt die Theilung ein."

The positive evidence upon the question from observations of normal cell division is thus rather scanty, though doubtless some additions might be made by a further study of the literature.

From both experimental evidence and observation of normal division the only conclusion possible is, that in some organisms the spindle *does* take a position in the greatest axis of the protoplasmic mass, apparently without regard to other factors, while in other cases the position of the spindle is determined by other factors, without regard to the form of the cell.

The result is at first thought not very satisfactory, but this is not the only organic phenomenon with regard to which such a conclusion must be drawn. A few examples will make this clear.

Stahl ('85) found that the direction of the first cleavage in the spores of Equisetum is determined by the direction of the infalling rays of light. A general statement of the effect of the direction of the light rays on cell division would take a form similar to the unsatisfactory conclusion above given for the shape of the cells.

If we leave cleavage and take up other growth phenomena, such as the direction of growth of plants and animals, such a conclusion would be reached with regard to almost every agent that ever exercises a determining power. The effect of light is to cause certain parts to grow towards it, others to grow away from it; while in other organisms the direction of growth is not affected by it at all. The same is true of gravity, of heat, and of various chemical and physical agents. (See the extended list of such cases given by Herbst, '94.) In all these cases we are dealing with *reactions to stimuli*. It is only when we attempt to make one of these agents the *only* determining factor, and expect to see it act always in the same way, as a simple mechanical cause, that our result becomes unsatisfactory.

It is evident that in this question of the relation of the form of cells to the direction of cleavage, we are dealing with a problem of a nature similar to those which I have briefly stated above. Some organisms are so constituted as to react to rays of light by growing toward them, others are not. In the same way, some cells or nuclei are so constituted as to react to the influences determining form by bringing the spindle into the longest axis of the protoplasmic mass; others are not. In each case the result is due to a reaction to stimulus, or to an action of similar nature, and not to a simple mechanical action of the agent.

In almost or quite all cases of reaction to stimuli, the result may be shown to be the accomplishment of a certain end that is of importance for the existence of the organism. The immediate explanation always takes a teleological form. It is not difficult to perceive a teleological aspect of this tendency of many cells to divide with the spindles in the longer axes. In cases where the purpose of cell division is merely to double the number of cells without alteration of form, division with the spindle in the longer axis is obviously the simplest method. A consideration of what would be the result if the opposite method prevailed shows this clearly. By continued division of a cubical cell and its products with the spindle in the shortest axis, a series of flat plates would be produced; every cleavage would bring about a greater modification in the form of the resulting cells, and cells of such form would undoubtedly be very inconvenient for the purposes of the organism. Continued division with the spindles in the long axis would result, on the other hand, in the production of cells of the same form as the parent cell. It is not remarkable, therefore, if in many cases the cells are so constructed as to respond to a change of form by a corresponding change of position of the spindle, so that the resulting cells shall be as nearly as possible of the form of the parent.

But mere increase of the *number* of cells without change of form is not the only object which can be brought about by cell division, and when other purposes are to be accomplished the cells are so constituted as to react in a different manner. Thus, I have shown that in Asplanchna divisions of cells with spindles in the short axis is the method by which is brought about the continued extension of cells in one direction, with consequent gastrulation and a later invagination of ectodermal cells. In Amphitrite, where, according to Mead, the division in the germ bands is with spindles in the short axes, this method of division is perhaps necessary to bring about the elongation of the germ bands, and the same is doubtless true in the germ bands of Crustacea. It is of course not necessary, nor is it probable, that in these cases the position of the spindle in the short axis is a reaction to the form of the cell; more probably the position of the spindle is determined without reference to this, as I have endeavored to show for the cells of Asplanchna.

The conclusion to which I have come is therefore similar to that maintained by Braem ('94), except that he seems to imply that the purpose of the cleaving cells is always the same, viz. to gain free space for the development of the products of division, whereas it appears to me that the facts indicate that the ends to be accomplished may be various, and the means by which they are brought about equally varied. This brings us to a nearer consideration of Braem's view.

(3) *Braem's theory of least resistance.* (Compare page 5.) — As just stated, so far as Braem's view is teleological, I must agree with him; but in so far as he seems to restrict his teleology to the accomplishment of a single purpose, — the attainment of the freest space for development, — it seems to me that the facts are against him. Certainly the principle of "least resistance" does not aid in understanding the cleavage of Asplanchna, where a large number of the divisions take place in what must be considered the direction of *greatest* pressure. Examination of the figures will show that, as a rule, the blastomeres in the resting stages are much flattened dorso-ventrally and extended laterally, as if subjected to great pressure; nevertheless, as shown in detail in the descriptive portion and in the discussion of Hertwig's law, when division takes place it is very frequently with the spindles in the dorso-ventral axes. The cleavage in this direction seems to have a purpose, but that purpose is not the gaining of the freest space for the development of the products of division, but the accomplishment of the process of gastrulation.

Since Braem's principle is confessedly teleological, it was probably not intended to be rigidly applicable to all cases; indeed, the author states as much. But in view of the frequent departures from the principle of least resistance, it appears necessary that the object of any given method of cleavage should be judged in each case for itself; so that Braem's contention that his principle is fitted "den Verlauf der normalen Furchung in wesentlichen Punkten zu erklären" must be considered unsuccessful. It may, by calling attention to one method in which cells react to a stimulus, "explain" the cleavage of some cells in the same sense that the growth of the stem and root of a plant may be said to be "explained" by saying that the protoplasm of one is so constituted as to react to light by growing toward it, the other, on the contrary, so as to react by growing away from it. But in other organisms the determining stimuli are of an entirely different nature, and the "explanation" must be sought anew for every organism.

I am of course fully aware that the view here put forth, that the position of the spindle must be interpreted teleologically, and in many cases as a reaction to stimulus, is not an "explanation." But I see no *a priori* ground for expecting a simple mechanical explanation for the direction of cell division, any more than we should of the direction of growth of a plant. As a matter of fact, the phenomena show that such an explanation is not at present possible for either.

(4) *Roux's theory of a compromise between the tendency immanent in the nucleus and the tendency due to the form of the protoplasmic mass.* (Compare page 6.) — As remarked above, this theory is not *definite*, in the same sense as the three foregoing, inasmuch as one of its factors — the immanent tendency of the nucleus — is of an entirely unknown character. From the foregoing description and discussion it is evident that I must agree fully with this conception. The further question comes, In how far do the phenomena in Asplanchna lead to a recognition of the second factor, — the tendency due to the form of the cells? It is evident that the form of the cell does not determine the main features of the direction of division, — the question as to whether the spindle shall be dorso-ventral or lateral in direction. But are there subordinate features in which the form of the cell does affect the position of the spindle, as held by Roux? In other words, does the spindle always lie in either the long or the short axis of the cell, and never oblique to both?

An examination of the figures will show that in the large majority of cases the latter question is to be answered affirmatively. In the earlier stages, before the great changes in the positions of cells have occurred, the dorso-ventral axis of the egg commonly coincides with either the greater or the lesser axis of the cells, so that from these cases no evidence on our question can be gained. But in later stages the crowding together of the cells results in greater or less alteration of the cell axes in relation to the axes of the egg. In Figure 68 (Plate **8**), for example, the long axis of the cell $d^{8.11}$ is not parallel with that of $d^{8.12}$; the two are wedged apart dorsally by the cell $d^{8.17}$, so that neither one points exactly to the animal pole of the egg. The two spindles are likewise at an angle with each other, and lie in the long axes of the cells, instead of exactly dorso-ventral. The same is true of the cells $c^{7.7}$ and $c^{7.11}$ in the same figure. Again, in Figure 74 (Plate **9**) the spindles of $d^{8.17}$ and $d^{8.19}$, though in general direction dorso-ventral, form an angle with each other, the one in $d^{8.17}$ being modified in position so as to lie in the longer axis of the cell, while the one in $d^{8.19}$ lies in the shorter axis of the cell. In the entoderm the same thing is strikingly true of the spindles shown in Plate **9**, Fig. 79, and Plate **10**, Fig. 83, for there the spindles form various angles with one another, all lying in the longer axes of the cells.

But these cases do not *necessarily* lead to the view that the form of the cell modifies even slightly the position of the spindle. It is possible that in each cell the axis of the spindle is determined otherwise, so that alteration of the *position* (not form) of the cell necessarily produces an alteration in the direction of the spindle. Thus in Figure 68 (Plate **8**), if the cells $d^{8.11}$ and $d^{8.12}$ are ellipsoids of fixed form in which the two ends of the spindles have predetermined positions, in case the cells are forced apart, as in this instance by $d^{8.17}$, the ends of the spindles will be forced apart to the same degree. Though we know that the cells are not ellipsoids "of fixed form," yet we also know that *the form of the cell is greatly influenced by the direction of the contained spindle.* It is possible that the cytoplasm of the cell tends to group itself symmetrically about the contained spindle, so that the direction of the spindle is the primary factor, the fact that it lies symmetrically in one of the axes of the cytoplasmic mass being a secondary result. This becomes very probable when we examine from this standpoint the change of form of the very irregular cell $d^{6.8}$, shown in Plate **5**, Fig. 37 (surface view), and Fig. 38 (section), before the formation of the spindle, and in Plate **7**, Fig. 54 (surface), and Plate **6**, Fig. 48 (section), after the formation of the spindle. In this cell, before the formation of the spindle, the shape is so irregular that it is not possible to distinguish a definite "short axis," and no plane would divide the cell into symmetrical halves. But as the spindle is formed, the cytoplasm groups itself symmetrically about it, until just before division the spindle lies in the shorter axis of the cell, and a plane including the long axis of the spindle and the centre of the egg would divide the cell into symmetrical halves.

If this be the true explanation, then the elongation of the cells in Plate **10**, Fig. 83, in various directions, is due to the previous alteration of the predetermined spindle axes. And certainly the elongation is not greater than is naturally the result of the position of the spindle, as has been shown for other cells.

This view is strengthened by the fact that in certain cases, where apparently it is impossible for the cytoplasm to group itself symmetrically about the spindle, the latter takes a position which is oblique to the axes of the cell. Such a case is shown in the entodermic cell $d^{7.1}$ of Figure 64 (Plate **8**); the spindle lies in neither the longest nor the shortest axis of the cell, but oblique to both.

It must be said, therefore, that the cleavage of Asplanchna gives no positive evidence of any effect of the form of the cell upon the position of the spindle; on the contrary, the evidence on the whole is decidedly against it. *This, of course, cannot be generalized and applied to other organisms,* as on the other hand the observed conditions in other organisms are not capable of giving generalizations which must hold for Asplanchna. Generalizations in the field of reaction to stimuli, which includes a very large proportion of organic activities, are exceedingly unsafe and are justifiable only after exhaustive examination of the phenomena; but in these matters scarcely more than a beginning of such an examination has been made.

(5) *Heidenhain's problem of a definite angle of rotation.* (Compare page 6.) — This certainly cannot be held to have any especial significance for the cleavage of Asplanchna; it is proposed by Heidenhain as applicable to systems of tissues in the later stages of organisms. I may point out some facts which bear upon the "problem."

In the cleavage of Asplanchna, the angle which a given spindle makes with the preceding spindle may be either 0 or 90 degrees; but it is generally either one or the other, not some angle intermediate between these.

The time and method of rotation, when rotation occurs, cannot be said to be "gesetzmässig." Commonly the asters come to lie on opposite sides of the nucleus before the rotation begins, but in the cells $d^{6.1}$ (Fig. 42, Plate **5**) and $d^{6.8}$ (Fig. 37) the change of position begins at the same time with the division of the asters, so that by a separation of the asters accompanied by motions not in a straight line, the same result is attained that would otherwise be produced by a rotation *after* the asters were fully separated.

In the larger cell at the two-cell stage, the change of position of the nucleus and spindle is not due to a rotation after the two asters are formed, but to a change of position of both nucleus and aster before the aster has begun to divide.

In $d^{4.2}$ (Plate **1**, Fig. 7) the asters and nucleus at first rotate into a position which is not the final one, so that subsequently rotation in a different manner is necessary to bring the spindle into its definitive position (Plate **2**, Fig. 16).

In $d^{6.3}$ (Plate **5**, Fig. 37, Plate **6**, Fig. 46, Plate **7**, Figs. 53 and 54) the definitive position taken by the spindle is at right angles to that of the preceding spindle, so that the simplest method of formation would be the natural separation at right angles to the axis of the preceding spindle, as commonly takes place in such cases. But, owing apparently to the peculiar form of the cell (Plate **5**, Figs. 37 and 38, surface and section), the asters separate obliquely, taking up a position such that the line uniting them is parallel to the axis of the preceding spindle; and the definitive position is reached only by a later rotation through an angle of 90 degrees (Plate **7**, Figs. 53 and 54).

There is thus no regularity about the *method* by which the asters come to occupy their definitive positions at the ends of the spindles. Apparently *the early position of the asters is influenced or determined by the mechanical conditions within the cell,* whereas the later position of the spindle is largely independent of such conditions.

On the other hand, in the divisions of the ectoderm of Asplanchna, there *is* a regularity in the final angle between the axes of two successive spindles in any given "layer" of cells, the second spindle being in *all* the cells of a given layer either parallel with or else at right angles to the preceding spindle. It is thus quite possible that there may be whole systems of tissues where there is such a regularity. But the fact that the definitive position may be reached by such various means renders the phenomenon of little significance for a mechanical theory such as that presented by Heidenhain.

(6) *Sachs's view that the walls separating the cells meet one another at right angles.*— An examination of the figures, especially the sections, shows that the condition above stated is not generally complied with in the cleaving cells of Asplanchna, so that it is not necessary to enter upon a discussion of this view. Neither is the regular alternation of spindles

at right angles to one another — a theory often attributed to Sach — the rule in Asplanchna.

(7) *Rauber's theory, that the asters of the different blastomeres exercise an attraction for one another, thus influencing the direction of the spindles.* — There appears to be nothing in the cleavage of Asplanchna that would lead to such a conclusion as the above. Any attempt to explain the relative positions of asters in the different cells — in such a case, for example, as is shown in Figure 39 (Plate **5**) — by the theory of attractions and repulsions among the asters, will be found to lead to purely arbitrary assumptions as to which asters exercise attractions on others and which do not; any general statement of a positive nature will be found to be inconsistent with the facts. Moreover, the irregularities in the migrations of the asters, to which attention was especially called in the discussion of Heidenhain's "Problem of a regular angle of rotation," can with difficulty be harmonized with such a theory.

(8) *Braem's principle of equal resistance at the two ends of the spindle.* — This principle is discussed in connection with the question which immediately follows.

B. *What determines the Equality or Inequality of Cleavage Products?*
C. *What determines the Rate of Cleavage?*

Owing to the similarity of the factors supposed to determine the relative size of the cleavage products and the time of cleavage, it will be well to consider together the foregoing questions.

In regard to the first question, we have (1) the theory of Hertwig, stated on page 8, that the spindle tends to take a position in the middle of the mass of protoplasm in which it is contained, and (2) the "principle of like resistance at the two ends of the spindle," set forth by Braem, and stated on page 7. Since these two theories lead to similar results, they may be considered together.

It certainly does not aid in understanding the cleavage of Asplanchna to assume that "der Kern von vornherein das Bestreben hat, sich gleichmässig nach beiden Seiten hin auszudehnen und somit auf eine äquale Zelltheilung hinzuwirken" (Braem, '94, p. 345), nor that "der Kern stets die Mitte seiner Wirkungssphäre einzunehmen sucht" (Hertwig, '93, p. 172). No differentiation into more and less yolk bearing regions is visible. Yet the first cleavage is unequal, and the second equal in one cell and unequal in the other; the third likewise shows both methods, with a preponderance of equal divisions; and in the fourth all the divisions are again unequal. In no cleavage is there throughout an equality in the size of the two products in all the cells. The two assumptions above stated are strikingly contradicted, not only in the formation of the polar cell (Fig. 1), but also in the fifth (Fig. 35, Plate **5**) and sixth (Fig. 49, Plate **6**) cleavages of the entodermal cell ($d^{5.1}$ and $d^{6.1}$), and in the division of the entodermal cell $d^{8.2}$ (Fig. 80, Plate **10**). In these cases the spindle moves against one side of th cell, and there a small vesicle is produced, the two products of cleavage being excessively unequal. The contradiction is emphasized by the fact that in the fifth and sixth cleavages of the entodermal cell (Figs. 35, 48, and 49), although there is a concentration of yolk in one region of the cell, it bears no significant relation to the position of the spindle, and by the further remarkable fact that in the next or seventh cleavage (Figs. 64 and 65, Plate **8**), this yolk cloud passes into the *smaller* blastomere.

When one considers the method of formation of polar cell in *all* eggs, and the almost unlimited range in the comparative size of cells resulting from cleavage in a great number of organisms, the grounds for the generalization, that "der Kern stets die Mitte seiner Wirkungssphäre einzunehmen sucht," are difficult to comprehend, at least so far as they relate to the dividing nucleus. As a statement of fact for specific cases, with the word "stets" omitted, I have no fault to find with it; but as a generalization it is, so far as it relates to the dividing nucleus, meaningless. The position of the spindle within the cell must be considered to be related to the purpose of the ensuing division. It is of course probable that in many cases the primary purpose is to divide the formative protoplasm equally between the two products, and this may determine the position of the spindle in, for example, the first, second, and third cleavages of the frog's egg. But what determines the position of the spindle in the two divisions immediately preceding these, — in the formation of polar cells? It seems to have been generally overlooked, even by those who have pointed out that polar-cell formation is cell division, that the formation of polar cells must be reckoned with in any general theory of cell division.

An examination of the cleavage of many invertebrates shows that, as in Asplanchna, equal cleavage is no more the rule than unequal cleavage, even where there is no corresponding differentiation into yolk bearing and purely protoplasmic regions. The statement that the dividing nucleus tends to take a position in the middle of its sphere of action is true, if we consider the "middle of its sphere of action" to be, as it actually is, the point where it divides. But with this interpretation the statement is of course utterly without significance. The real question is, What determines the point where it divides? Why in Asplanchna does the middle of the sphere of action in the fourth and seventh cleavages of the large ventral entoderm cell lie in such a position (Fig. 16, Plate **2**, and Fig. 65, Plate **8**) as to divide the cell into parts which are only slightly unequal, while in the intervening fifth and sixth cleavages (Fig. 35, Plate **5**, and Fig. 49, Plate **6**) the middle of the sphere of action is at the periphery of the cell? Why at the first cleavage of the frog's egg is the middle of the sphere of action in the centre of the mass of formative protoplasm, whereas at the divisions immediately preceding — the formation of the polar cells — it is at the periphery of the egg?

In regard to the question what determines the comparative *rate* of cleavage the case is similar. In view of the recent discussion of these two questions — the inequality of cleavage and the rate of cleavage — in the works of Kofoid ('94, p. 196), McMurrich ('95), Lillie ('95, p. 45), zur Strassen ('95), Ziegler ('95), and others, it seems scarcely worth while to insist upon the fact that the rate of cleavage and the equality or inequality of the products are related to the future morphogenetic processes, and show in many cases no relation whatever to accumulations of yolk. Yet the so called laws, according to which these matters are determined by the distribution of yolk, are repeated in O. Hertwig's text-book on the cell (Hertwig, '93, pp. 174 and 180), and reaffirmed in the latest edition of his treatise on embryology (Hertwig, '96, pp. 67 and 84).

In Asplanchna the main facts in regard to the sequence of cleavage are as follows.

(1) The order of cleavage is, within very narrow limits, *constant*. If a number of eggs are taken showing successive stages of the division of any given cell, the series will show corresponding successive stages in the nuclear history of the other cells.

(2) There is a typical order for similar cells of different quadrants of the same egg. This order is *D*, *C*, *B*, *A*.

(3) There is a marked general tendency for larger cells to divide first. In every case where two cells of the same origin are of different size, the larger divides before the smaller.

This is a very general phenomenon, as has been repeatedly pointed out of late, even in cases where the larger cell contains the greater amount of yolk. The "law" recently formulated by Hertwig ('96, p. 67), that "die Schnelligkeit der Furchung proportional ist der Concentration des im Theilungsstück befindlichen Protoplasmas," must be considered — like the corresponding one in regard to the equality or inequality of cleavage products — as an example of the immature generalizations to which embryology has fallen heir through the accidental circumstance that the amphibian egg was for a long time the chief object for the study of cell division. It seems to be exceedingly difficult to grasp the fact, every day becoming more evident, that because a statement is true for the eggs of some of the lower vertebrates it does not follow that it must be true for the cells of all organisms.

(4) The rhythm of cleavage has an important relation to the other processes of morphogenesis. If cleavage took place coincidently in all the cells of the egg, the latter still retaining its form, there could apparently be no "rotation" of the cells upon one another, and consequently no gastrulation. The tension in all directions would be the same; none of the cells would be moulded to fit the extension of neighboring cells, and all would retain approximately the positions held at the beginning.

It would perhaps be possible to carry this into detail, and show that the *earlier* cleavage of the large ventral cell, leaving it in a resting condition, and therefore *plastic* (as indicated by a comparative study of the forms taken by resting and by dividing cells) when the other cells divide and extend, is directly favorable to gastrulation.

The question as to the factors determining the time and the equality or inequality of cleavage, it will be seen, does not at present admit of any direct simple answer. Cleavage is a part of the process of morphogenesis, and its rhythm and other features are related to the nature of the form to be produced.

D. *As to Differentiation accompanying Cleavage.*

The facts bearing upon this question to be derived from the observation of the early development of Asplanchna are few in number. The principal phenomenon to which attention must be directed is that of the segregation and migration of the cloud of granules, described on pages 25, 30, 37, and 54. As will be remembered, a concentration of granules begins in the ventral region of one of the cells at the eight-cell stage (Fig. 7) and becomes more and more marked as successive cleavages take place, till a well defined cloud of very large and distinct spherules occupies the anterior and ventral margin of the cell $d^{5.1}$ in the stages immediately succeeding the sixteen-cell condition (Fig. 32, Plate **4**, and Fig. 48, Plate **6**). Then occur the remarkable migrations shown in

Figures 49, 50, 51, and 64 (Plates **6** and **8**), until the cloud of granules is enclosed at the seventh cleavage in the smaller of the two entoderm cells (Fig. 65). This whole process shows clearly that other changes of a striking character are taking place at the same time as the division of the egg into smaller portions; evidently cleavage is not a mere separation of the egg into smaller masses, each similar to the other and to the original egg. The final destiny of this granular mass is not known, but such a peculiar and well characterized phenomenon as it exhibits cannot be considered meaningless.

The differentiation in this instance is of the kind admitted by Driesch (see page 9), in that it is cytoplasmic in nature. It is not, however, a direct consequence of the original distribution of materials within the egg; the migrations of the granules show that processes are taking place in the cytoplasm that are only indirectly connected with cell division.

We have in this case a distinctly visible differentiation accompanying cleavage. Certain other phenomena give evidence that there are likewise invisible differentiations accompanying the process.

At the division of the second "layer" of ectodermal cells in the sixth cleavage, shown in Figure 55 (Plate **7**), the two rows of cells $a^{7.5}-c^{7.5}$, $a^{7.6}-c^{7.6}$, $a^{7.7}-c^{7.7}$, and $a^{7.8}-c^{7.8}$ are produced. The cells of these two rows, as shown in Figure 61, are of the same size and the same form, having similar relations to the surrounding cells and to the axis of the embryo. Yet, as has been repeatedly stated, all the cells of one row divide meridionally and equally with spindles in the long axes, the cells of the other row equatorially and unequally with spindles in the short axes. What causes this difference?

The difference must, of course, be due either to a different stimulus from the outside, or to a different structure of the cells. The problem may be expressed clearly in this way: If one of the cells of the more dorsal row, as $a^{7.8}$ (Fig. 61), could be removed and placed in the position now occupied by $a^{7.7}$, in the more ventral row, would it change its method of division? That is, would it cleave equatorially and unequally, with its spindle in the short axis, like the other cells of the ventral row, instead of meridionally and equally, with its spindle in the long axis, as it actually does?

There is, of course, no way of answering this question directly. It scarcely appears probable, however, that there is such a difference in the influences affecting the two cells as to cause so fundamental a difference in the cleavage. And if there is not, the only alternative is, that there was a qualitative division, nuclear or cytoplasmic, at the preceding cleavage.

This conclusion is, of course, speculative, but the history of the cloud of granules directly proves that in Asplanchna the cleavage is accompanied by differentiation.

The recent experimental evidence, showing that in certain organisms the cleaving cells in early stages all possess the inherent capacity to produce an entire animal, has led to a rather widespread impression that cleavage has been shown to be a process of little or no significance, being merely a quantitative division of a mass into smaller masses of a similar nature. This view apparently receives confirmation from generalized statements of the results of such experiments; for example, the following from Driesch ('94, p. 69): "Es liegt also nach allem gesagten in der That kein Grund vor, in der Furchung etwas anderes als reine Zellteilung zu sehen; ja die Gleichheit der Furchungskerne ist direkt durch Versuche bewiesen." A summary of the evidence which has been adduced in regard to this matter shows that such a statement as the above conveys only a small part of the truth and must lead to error unless carefully interpreted. The evidence that cleavage is accompanied by differentiation may be summarized as follows.

(1) It is directly proved by observation that in certain cases the *nucleus* differs in structure in different blastomeres in early cleavage stages, and that *this differentiation is correlated with a different fate of the differing cells.* This Boveri has shown for Ascaris megalocephala (Boveri, '94), and Meyer ('95) for certain other nematodes.

(2) It is directly proved by observation that in certain cases the *cytoplasm* of the different cleavage cells in early stages is of a different structure, reacting differently to chemical reagents, and *this difference is correlated with a different fate of the different cells.* Thus, in the sixteen-cell stage of Nereis, "the somatoblast can always be recognized at a glance" from its different color (Wilson, '92, p. 390). A similar fact has been shown above in regard to the cytoplasmic differentiation in Asplanchna, but here I have not been able to determine the fate of the cell which receives the differentiated granules.

(3) It has been shown that in many cases the different blastomeres of early cleavage stages give rise to definite structures in the adult. This fact in itself of course admits of two interpretations, but taken in connection with the facts stated under (1) and (2) it becomes of great significance.

(4) It has been shown experimentally, that in some organisms sepa-

rated blastomeres give origin to *parts* of the embryo only. The most complete and satisfactory cases are those of ctenophores as described by Chun ('92) and confirmed by Driesch und Morgan ('95), and of the gasteropod Ilyanassa, by Crampton ('96).

It is difficult to conceive how a more complete demonstration could be possible, that cleavage is accompanied in many cases by differentiations which are not expressed by the phrase "reine Zellteilung," and that these differentiations are of the utmost significance for the future development of the organism. Any amount of evidence that in *other cases* there is no differentiation cannot in the least shake confidence in this demonstration

2. GASTRULATION.

In addition to the problems bearing directly upon cleavage, the plan of the present work included a study of some of the later morphogenetic processes, affecting *masses* of cells and leading to the differentiation of organs, in order to determine the relation of cleavage to these. Of these processes, gastrulation and the ensuing invagination of ectodermic cells to form the pharynx (Zelinka, '91) were studied. These are in reality parts of a single process, so that they may be treated of together under the title of Gastrulation.

In regard to the relation of cleavage to gastrulation, the result is evident from the account given in the descriptive portion of this paper. No separation of the two processes is possible; gastrulation is an accompaniment and a consequence of cleavage. At the passage from the four-cell stage to the eight-cell stage begins a displacement of the blastomeres; this displacement, or "rotation," continues in later cleavages in the same direction, and is still in operation at the latest stage examined, when it is no longer possible to follow the development cell by cell. As one of the phases of this displacement during cleavage, the large ventral cell of quadrant *D* gradually moves inward, followed later by a similar transference of the ventral cells of the other quadrants to the inside. The entire process has been followed step by step in the descriptive portion of the paper, so that it is not necessary to go into details here. In its general features the process is as follows. As the ectodermal cells begin to pass into the karyokinetic condition, they withdraw their more internal parts and increase in surface extent. The egg as a whole retains its form and size, so that the withdrawal of the internal parts of one cell necessitates an inward movement on the part of others; the result is a gradual inward migration of the ventral cells.

The inward movement of the large cell of quadrant *D*, rather than that of the other cells, seems due to several causes: — (1) The inequality of the cells. The large ventral cell having a much greater radius of curvature has less surface tension, and therefore may more easily change its form. (?) It thus yields to pressure, and fits itself to the changing form of the smaller cells. These are thus able to creep over it, as it were, and surround it. The greater quantity of cytoplasm in the entoderm cell as compared with the size of the spindle seems also to result in less change of form at the time of karyokinesis. The large cell, in virtue of its mere *size*, conducts itself on the whole *passively* with relation to the more active smaller cells. (2) The sequence of cleavage is possibly connected with this. At a given cleavage the large cell divides first, so that, when the karyokinetic stretching of the other cells takes place, the entodermic cell is in a resting condition, and therefore passive. (3) The direction of the spindles, which is prevailingly dorso-ventral, results in a continued dorso-ventral extension of the cells, so that invagination would naturally take place at one of the two ends. The developing egg may be likened to a fountain in which there is an upward movement within, an outflow above, at the animal pole, and a downflow about the periphery.

The enclosure of the large ventral cell of quadrant *D* is what has been considered gastrulation proper by Zelinka and Tessin, only the products of this cell being spoken of as *entoderm*. But after this enclosure is complete the process continues, unchanged in character, the ventral cells of the other quadrants following that of quadrant *D* to the inside of the embryo, as shown in Figures 76–79 (Plate **9**).

A necessary condition for all this displacement is of course the retention by the egg as a whole of its form and outline. If the blastomeres should separate and project above the general level, in the manner that is common for the cells of mollusks (see the figures of Unio by Lillie, '95) at the time of karyokinesis, no compensating inward movement of the other cells would be necessary, and apparently therefore gastrulation would not take place. The retention of the regular form appears thus to be of the highest importance for the development, and the question arises as to how this form is preserved. As previously stated, no membrane is visible; and any uniform force, such as surface tension or a centripetal attraction, would produce a spherical instead of an ellipsoidal form. The development of the egg proceeds as if it were enclosed in a rigid mould of oval or ellipsoidal form, so that the contents of the mould are rotated, while the form is retained. The retention of this shape seems to me inexplicable on any simple mechanical ground; the form at this stage apparently must be judged from the same standpoint as that of the adult, which no one would attempt to refer to a simple mechanical principle. Of course the assumption of the presence of a non-elastic non-extensible membrane of ellipsoidal form, which later becomes elastic and extensible, would explain the retention of the shape, and is open to any one who chooses to make it. But several facts speak against this view, aside from the general improbability of the existence of a membrane of such peculiar and changing qualities: — (1) The negative evidence that no such membrane can be demonstrated in preserved material. (2) In the sea-urchin and in Amphioxus, as shown by Driesch ('93) and Wilson ('93), development takes place as well when the membrane is removed as when present. This of course does not show that the same is true for Asplanchna, but it does show that the importance of the egg membrane has been overestimated for some cases. (3) In the rotifer Callidina, investigated by Zelinka ('91), the egg is of the same form as in Asplanchna, yet the cells sometimes put forth short amœboid processes, which of course would be impossible with a close membrane. (4) In another rotifer, Melicerta ringens, the egg is not a regular oval or ellipsoid of rotation, but one side is flattened while the other is curved, and this irregular form is retained during the shifting of the blastomeres, as is the case in Asplanchna (see the figures of Zelinka, '91, and of Joliet, '83). Such a form would not be preserved even by such a non-elastic membrane.

The facts given under (3) and (4) seem to me to render entirely inadmissible the explanation that the form of the egg in Asplanchna is due to the presence of a membrane, since this would leave the exactly similar phenomenon in the related forms Callidina and Melicerta without explanation.

The factors concerned in gastrulation may be summarized as follows: —

1. The form of the egg.
2. The change in the form of the cells at cleavage.
3. The direction of cleavage.
4. The inequality of the cleavage.
5. The sequence of cleavage. (?)

The process of which gastrulation is a part begins with the third cleavage, and continues through all the period in which it is possible to trace the development cell by cell, and apparently much later.

The process of gastrulation as above described for Asplanchna is similar to the method briefly set forth by Ziegler ('95, p. 402, note) for

Rhabditis nigrovenosa: "Ich stelle mir die Mechanik des Gastrulationsvorganges so vor, dass die Ektodermzellen nach ihrer Theilung sich abflachen und in Folge dessen ausbreiten; dabei schieben sie die Mesodermzellen über die Entodermzellen herüber. Es kann dies um so eher geschehen, da die Mesodermzellen zum Zweck der Theilung sich kugelig zusammengezogen und dabei an die Oberfläche des einschichtigen Epithels emporgehoben haben."

On the other hand, zur Strassen ('95 and '96), in his careful studies on the early development of another nematode, Ascaris megalocephala, came to an entirely different conception of the factors at work in the displacement and extension of the ectoderm cells. Zur Strassen holds that four cells of common origin constitute an "elementary mechanism," the two pairs of which attract each other in such a way as to bring about the movements which actually occur. The interaction is thought of as something having the nature of the "cytotropism" of Roux ('95). It is not necessary to discuss the matter further here, since there is no occasion to call in any such action in the gastrulation of Asplanchna. Doubtless the inter-attraction of cells exists in the rotifer, as elsewhere, but it seems to have no determining significance for the movements which take place.

3. General Considerations.

I shall next take up certain general considerations upon the mechanics of cleavage and development, which do not naturally fall under the discussion of any of the theories above considered, together with a general review of the results gained.

The egg of Asplanchna at the four-cell stage might be compared to the egg of an echinoderm, with the exception that one of the four blastomeres is much enlarged, and of a different form from the others. What variation in the cleavage will be induced by the differences in form?

As we have seen, the form of the cell in Asplanchna does not affect the direction of the spindle at cleavage. Indeed, the most characteristic feature of cleavage in Asplanchna is *difference in form and size of the blastomeres*, coupled with *identity in the direction of spindles*, in cells having the same general relations to the axes of the embryo.

In a closer consideration of the factors determining the position of spindles, it is evident from the phenomena described that the question is not a simple one, but must be resolved into (1) what determines the direction of separation of the asters; (2) what determines the position of the asters before the spindle is formed; and (3) what determines the movements of the asters into the definitive position occupied by the spindle.

(1) and (2). In regard to the first two questions, two general facts are worthy of notice.

First, there is a tendency, other things being equal, for the newly formed asters to separate at right angles to the axis of the preceding spindle, and in such a way that the asters are not to be distinguished as deep and superficial, but either as right and left, or dorsal and ventral. No explanation for this fact is apparent, and it is not in every case true. Thus in the two-cell stage the nucleus and aster in the large cell migrate to the right before the aster divides, and the separation of the two newly formed asters is not in a plane at right angles to the axis of the preceding spindle.

Secondly, the position of the asters before the formation of the spindle may apparently be modified by the simple mechanical conditions surrounding them. Thus, in the cell $d^{4.2}$ (Fig. 14, Plate **2**) the asters are modified in position almost immediately after they begin to separate, so that very soon we actually have in this cell the condition which may be considered least typical, — an inner and an outer aster (Fig. 7, Plate **1**). In the thin cell $d^{6.3}$ (Figs. 37 and 38, Plate **5**, and 46, Plate **6**) the form of the cell apparently operates to cause the two asters to separate in such a way that almost from the first the line joining them has the same direction as the axis of the preceding spindle. Such facts give the impression that before the formation of the spindle the position of the asters is undetermined, and indifferent for the general structure of the cell, except that the two asters always lie on opposite sides of the nucleus.

(3) As the karyokinetic changes take place, the asters migrate into definite positions, apparently by a rotation of the whole complex composed of the nucleus and its two accompanying asters. This rotation is into a definite *position*, without regard to either the form of the containing cell, or the previous position of the asters; that is, the *end* to be gained is constant, while the *means* of gaining it vary. Thus, at the divisions to form the sixteen-cell stage, the line passing through the asters and nucleus rotates in $d^{4.1}$ from a lateral to a dorso-ventral position, and into the greatest axis of its cell (Figs. 11, 15, and 16, Plate **2**). In $d^{4.2}$ it rotates from a position in which one aster is central, the other peripheral (Plate **1**, Fig. 7), likewise into a dorso-ventral position (Fig. 16). In $a^{4.1}-c^{4.1}$ and $a^{4.2}-c^{4.2}$ all the axes rotate from a position of

lateral extension to a dorso-ventral position, and thereby *from the greater into the lesser* axes of their respective cells (Figs. 17–22, Plate **3**). In $d^{6.3}$ the asters do not separate at right angles to the previous spindle, as commonly occurs, but the line joining them is parallel to the preceding spindle, i. e. lateral (Figs. 37 and 38, Plate **5**, and 46, Plate **6**); later, by a rotation into the short axis of the cell, the dorso-ventral position is attained (Plate **6**, Fig. 48, Plate **7**, Figs. 53 and 54). It is not possible to refer these and the other changes described in the general account of the development to any simple factors. We can refer the changes in position of the asters, and consequent manner of cleavage, only to the structure of the protoplasm and the (molecular?) processes occurring within it.

The fact that the spindles take definite positions with relation to the axes of the developing embryo, but without regard to the form of the cells, seems to indicate that there is some influence governing the egg as a whole, which is related to its form, and that the position of the spindles is regulated by this. The determining factors in the position of the spindles would therefore lie, not within any given cell itself, but outside of it. But there are certain facts which seem to render this very doubtful. As discussed on pages 70, 71, in later stages the cells become displaced by the changes taking place during gastrulation, and there is a corresponding change in the position of the spindles; they are no longer either parallel with or at right angles to the dorso-ventral axis of the egg. This is shown especially in Figures 68 (Plate **8**) and 83 (Plate **10**). If a changed position of the cell with regard to the axis of the embryo results in a correspondingly changed position of the cleavage spindle, it seems to follow that the position of the spindle is determined *within* the cell.

I do not, however, consider this conclusion as well established. It still seems *possible* that the spindles are all placed with reference to some influence resulting from the axial relations of the egg as a whole, — though not necessarily in all cases either in the dorso-ventral axis or at right angles to it.

Comparison of the conditions in Asplanchna with those reported by other observers for other organisms shows that there are cases in which the *form* of the cell *does* determine the position of the spindle. In the same way we know that there are cases in which the direction of the infalling rays of light determines the position of the spindle (Stahl, '85). But the result is not universal for either agent, so that we must hold that the effect in both cases is of the nature of a reaction to stimulus.

The form of the cell and the direction of pressure cannot therefore be used in explaining in any general way the direction of cleavage, as proposed by Hertwig and Braem. The method of reaction and the purpose of the reaction must be determined for each class of cases in itself.

A consideration of the process of gastrulation leads, though from the opposite direction, to conclusions of a similar nature. The form and direction of cleavage are related to the later morphogenetic processes. Gastrulation is a result of the method of cleavage, — and the method of cleavage must be looked upon as adapted to the purpose of accomplishing gastrulation.

The relation of the form of cleavage to the later morphogenetic processes is shown in a different way in such forms as Nereis (Wilson, '92) and Unio (Lillie, '95), where it has been possible to show the exact relation of later organs to individual blastomeres. Cleavage in many cases is itself a direct morphogenetic process, the exact method of which can be referred to no more simple mechanical factors than can the characteristic form of the adult.

I do not of course wish to generalize this statement; it is evidently true for many forms, but may not be true for all. The evidence upon which a contrary view is sometimes maintained for certain forms, as the echinoderms, seems however inconclusive. The formation of the micromeres has been shown to be preceded by a differentiation in the cytoplasm (Morgan, '94), which would naturally lead to the conclusion that the micromere formation is a process having a definite signification for morphogenesis. But Driesch ('93) showed that the micromeres might be removed and a normal larva still produced. From this, however, it does not follow that the formation of micromeres is without definite morphogenetic significance, any more than it follows that the fundaments of limbs are of no morphogenetic significance because a normal larva results after the embryonic limbs have been removed from a young amphibian. The formation of micromeres apparently segregates a certain amount of substance which needs to be localized in a definite region. If this segregated material is removed, there is no evidence proving that similar material is not again segregated, at perhaps a later stage. As Roux insists, it is important to distinguish the normal method of development from induced development due to injury.

PART SECOND.—DISCUSSION OF MATTERS BEARING UPON THE MORPHOLOGY OF THE ROTIFERA.

Our knowledge of the development of the Rotifera is due chiefly to the work of Zelinka ('91). This author has given a full and careful description of the development of Callidina russeola Zel. from the egg to the adult form, with a briefer, but still extended, comparative account of the development of Melicerta ringens. Earlier works on the embryology of the Rotifera are due to Salensky ('72), Joliet ('83), Zacharias ('84), and Tessin ('86); but all of these works are incomplete and in many respects inaccurate, so that they have been almost completely superseded by the work of Zelinka. In discussing the development of Asplanchna I shall therefore restrict myself chiefly to a comparison with the results of Zelinka, drawing upon the accounts of other authors only where there is special occasion.

Since my work has been done primarily from the standpoint of cytomechanics, and not with regard to the morphology of the Rotifera, it has an entirely different aim from that of Zelinka. It thus results naturally that, in giving an account of the bearing of my studies on questions relating primarily to rotifer morphology, emphasis must be laid chiefly upon the points in which my results differ from those set forth in Zelinka's paper. The plan of my work required a more minute study of the cleavage than was demanded for Zelinka's purposes, and as a natural result I shall be compelled to criticise his account of the cleavage in regard to certain details. Furthermore, it will be necessary to show that Zelinka has been inconsistent in his account with regard to the place where the polar cell is formed, and hence is mistaken in his statement of the axial relations of the egg and embryo in the Rotifera. But all these are matters of detail, not affecting in any important way Zelinka's general conclusions, and I wish to say at the beginning that I fully appreciate the thoroughness and excellence of Zelinka's researches upon this difficult group, and make the criticisms and corrections contained in the following pages in no spirit of disparagement.

Asplanchna Herrickii de Guerne, the form upon which my studies have been made, is not closely related to any of the species of Rotifera whose development has been previously described. Callidina and Melicerta, investigated by Zelinka ('91), belong respectively to the groups Bdelloidea and Rhizota of Hudson and Gosse ('86). Rotifer and Philodina, studied by Zacharias ('84), belong also to the Bdelloidea. Brachionus, investigated by Salensky ('72), is one of the Loricata. Only Eosphora, upon which the work of Tessin ('86) was done, belongs, together with Asplanchna, to the swimming Illoricata. But even these two are widely separated, Eosphora possessing a foot and anus, like the majority of the Rotifera, whereas Asplanchna possesses neither.

The only work which has been done upon the early development of any species of Asplanchna is that by Leydig ('54) and Lameere ('90). The latter observed in the living egg of Asplancha Sieboldii the formation of the polar cell and the first and second cleavages, but did not carry his work further. Leydig ('54) observed the cleavage of the egg, and figured the four-cell stage in the same species, but does not give any detailed description. I know of no other work dealing with the early development of any species of Asplanchna.

I have examined the early stages of both Asplanchna Herrickii de Guerne and Asplanchna priodonta Gosse, of which the material collected in the Great Lakes contained about equal numbers. Asplanchna Herrickii was chosen for special investigation on account of the greater size of the embryos and adults. The development of Asplanchna priodonta was examined for comparison, and notes upon the embryology of this species have been given in connection with the fuller discussion of Asplanchna Herrickii.

1. Previous Knowledge of Asplanchna Herrickii.

It will be well to give here a brief review of previous references in the literature to the little known species Asplanchna Herrickii. It was first figured by Herrick ('84, Plate V.), and described in the explanation of plates as "flask-shaped Rotifer, hermaphrodite, with eggs and sperm," but no further description was given and no name was proposed. Herrick in a second paper ('85, p. 61) again mentions this form, but adds nothing to the description.

In 1888, Jules de Guerne ('88) reproduced Herrick's figure of the jaws, and on the basis of this held that the species was new, and proposed for it the name Asplanchna Herrickii.

Hudson and Gosse ('89) included Asplanchna Herrickii in their list of "doubtful species."

Daday ('92) admits Asplanchna Herrickii as a valid species.

Up to this time no observer since Herrick had reported finding this species. But in this same year, 1892, Wierzejski ('92) gave an account of its presence in Galicia, with a description, and figures of the jaws and the peculiar glandular organ which Herrick had mistaken for a testis. Again, in his Rotatoria (Wrotki) Galicyi, Wierzejski ('92^a) gives a figure of the entire animal, with special figures of the characteristic glandular organ, the trochal field, the jaws, and the excretory system, together with a brief description in the Polish language.

Asplanchna Herrickii was afterward reported by the author (Jennings, '94) as occurring in Lake St. Clair, and by Levander ('95) as occurring in the neighborhood of Helsingfors.

2. Development.

The unsegmented egg of Asplanchna Herrickii is similar in form to that of Callidina russeola, investigated by Zelinka, but slightly smaller, the maximum dimensions in Callidina being $130\,\mu$ by $90\,\mu$, while the maximum dimensions observed in Asplanchna Herrickii were $97\,\mu$ by $83\,\mu$.

No thick-shell "winter eggs" were ever observed by me in the specimens taken; possibly later in the fall these would have been found.

In regard to the details of the developmental process, reference must be made to Part First; the purpose here is merely to point out such observations as are of importance from the standpoint of rotifer morphology, noting especially any differences between my account and those of other writers.

A. Maturation.

As stated on page 13, the place of polar-cell formation in Asplanchna has a different relation to the axes of the egg from that ascribed to it by Zelinka in Callidina. In the ensuing discussion I shall, for convenience of comparison, use the orientation adopted by Zelinka; that is, what I have called the "macromere end" is anterior, the "micromere end" posterior. That side of the egg upon which later the blastopore is found, occupied in early stages chiefly by the quadrant *D*, is ventral, the opposite side dorsal. These terms have no constant relation to the terms of orientation employed in Part First.

As previously described and figured (Plate **1**, Figs. 1 and 2), the polar cell is formed near one of the ends of the ellipsoidal egg, and the place of formation is cut by the first and second cleavage furrows (Figs. 6 and 8). The same is true for Asplanchna priodonta.

In these two species of Asplanchna, therefore, the place of polar-cell formation is the same, with reference to the form of the egg, as that described by Lameere ('90) for Asplanchna Sieboldii, and by Zelinka ('91) for Callidina Leitgebii (p. 53) and Melicerta ringens (p. 117). In

Callidina russeola and Discopus synaptæ, according to Zelinka ('91, p. 53), the polar cell is formed almost exactly at one of the ends of the ellipsoidal egg, though a very little to one side. This difference is a point of little or no significance; an examination of Zelinka's figures (Taf. I. Figg. 1–5) shows that the polar cell in Callidina russeola occupies the same position with regard to the *axis of the first cleavage spindle* as it does in the three species of Asplanchna. Possibly the egg of Callidina russeola is forced by the shell to take such a form that the axes of the egg, as indicated by the first cleavage spindle, do not coincide with the apparent axes indicated by the shape. The place of formation of the polar cell, as might be expected, is correlated with the axis indicated by the cleavage spindle. After the first cleavage a rotation occurs in Callidina russeola, bringing the apparent axis into agreement with the real axis.

But with regard to the place of polar-cell formation in its relation to the orientation of the egg as shown by later development, a remarkable disagreement exists between the condition in Asplanchna Herrickii and Asplanchna priodonta on the one hand, and the description given by Zelinka of Callidina russeola on the other. The following is Zelinka's statement of the orientation of the egg of Callidina with relation to the place of polar-cell formation: —

"Es verdient hervorgehoben zu werden, dass von dem Augenblicke an, als das Richtungskörperchen gebildet wird, sämmtliche Richtungen im Räderthier-Eie orientirt sind. An dem Pol, in dessen Nähe das Körperchen austritt, finden wir später das Vorderende, am gegenüberliegenden das Hinterende, während die Fläche, in der es erscheint, zur Rückenfläche wird." (Zelinka, '91, p. 54.)

Accepting the later orientation of Zelinka, the above statement becomes accurate for Asplanchna Herrickii and Asplanchna priodonta if "Vorderende" and "Hinterende" are interchanged, and "Bauchfläche" is substituted for "Rückenfläche"; in other words, *the orientation of Asplanchna with reference to the polar cell is precisely the opposite of that of Callidina.* The statement for Asplanchna would read: "At the pole in the neighborhood of which the polar cell appears, we find later the posterior end, at the opposite pole the anterior end, while the surface on which it appears becomes the ventral surface." This statement, while correct if we relate the orientation of the animal simply to the *form* of the egg, as is done by Zelinka, contains one false implication. While that surface of the egg on which the polar cell is formed *does* later become the ventral surface of the animal, — the same form being retained to a late stage, — yet during the processes of development that part of the *material* of the egg immediately surrounding the region of polar-cell formation is moved to the posterior end of the egg, and, later across this and even upon the dorsal side. The same is doubtless true for the corresponding region (opposite the place of polar-cell formation, according to Zelinka) in Callidina. It marks the *animal* or *upper* pole in Asplanchna, lying at the opposite end of the egg from the blastopore during gastrulation, and is the common point of meeting for the blastomeres derived from the four quadrants of the egg. In Callidina russeola, according to Zelinka, the region where the polar cell is formed lies, not at the opposite end of the gastrula from the blastopore, but at the *dorsal margin of the blastopore,* and the cells of this region are later invaginated to form the fundament of the pharynx; the real animal pole of the egg lying at a distance from the point of polar-cell formation. The whole of Zelinka's general discussion of the early development of the rotifer egg is based upon this peculiar position of the polar cell. (See Zelinka, '91, pp. 132–135.) His general statement of the place of polar-cell formation is as follows: "Das Richtungskörperchen kommt an der dorsalen Seite des künftigen Embryo hervor, bei Melicerta dem späteren hinteren Pole näher, bei Callidina fast am späteren vorderen Pole des in beiden Fällen länglichen Eies."

The difference between our accounts is seen by comparing Figure 6 (Plate **1**) and Figure 8 (Plate **2**) with Zelinka's Figures 8, 9, and 10 (Tafel 1.). In the two-cell stage in Asplanchna (Fig. 6), when the smaller cell, $\overline{AB}^2$, is turned away from the observer, who looks down upon the polar cell, the spindle in the larger cell is seen to occupy such a position that the smaller product of the division of $\overline{CD}^2$ will lie to the *right,* and in Figure 8 this condition is shown to be realized when the division has taken place, the cell C^3 lying to the right of the polar cell. In Zelinka's figures, however, the small cell II (= C^3), derived from the division of the larger blastomere of the two-cell stage, lies to the *left* of the polar cell, when the same orientation is adopted; i. e. with the smaller blastomere ($A = \overline{AB}^2$) of the two-cell stage away from the observer. It therefore follows that, if the position of the polar cell is dorsal in Callidina, it must be ventral in Asplanchna, *using Zelinka's orientation.* Later stages show the same contrast. Thus Figures 13 and 14 (Plate **2**), representations of the eight-cell stage of Asplanchna, show the polar cell in the position already described, (the dorsal pole of the egg being directed toward the observer,) whereas Zelinka's Figures 15, 16, and 18 of Callidina show it at the opposite pole. Figures 19, 20 (Plate **3**), 38, 41 (Plate **5**), and 59 (Plate **7**) show the polar cell at later stages in Asplanchna, and demonstrate clearly that it lies at the animal pole of the egg, opposite the blastopore.

Moreover, a careful examination of Zelinka's own work shows that the general statement above cited cannot be considered correct for all Rotifera. His general conclusions are based throughout primarily upon his more complete study of Callidina, and in his "Theoretischer Theil" he seems to have overlooked the fact that in Melicerta the place of polar-cell formation observed by himself was, not only not near the anterior end, as noted in the above general statement, *but also not upon the dorsal side.*

The egg of Melicerta is described (Zelinka, '91, p. 115) as an elongated ovoid with a sharper and a thicker or blunter end; one side is cylindrical in form, so as to appear straight in profile, while the other is swollen, presenting in profile a convex outline. Zelinka says in regard to the orientation of the egg given by Joliet: "Da ganz richtigerweise das dickere End als das Kopfende, das dünnere als das Hinterende bezeichnet wird, sowie ferner dieser Autor dessgleichen richtig die vorgebauchte Fläche als die ventrale, die cylindrische als die dorsale ansieht, so wäre die Orientirung durch diese Form des Eies eigentlich erleichtert," etc. Now, in Zelinka's figures of the maturation stages of Melicerta (Taf. V. Figg. 74–76) the polar cell is clearly shown to be formed at this *convex*, and therefore *ventral* side. Furthermore, his description of the process of maturation is as follows: "Das Keimbläschen zeigt zuerst noch seine wohlbegrenzte sphärische Gestalt (Fig. 73) und liegt etwa in der Mitte des Eies, sodann wandert es, indem es lebhaft seine Gestalt verändert, gegen den hinteren Pol, wird zu einem halbmondförmigen Fleck mit gekerbten Rändern (Fig. 74), dessen Konvexität der *Bauchfläche* zugekehrt, *welcher es sich rasch nähert.* Knapp unter der Oberfläche zerlegen die Kerben den Kern in drei eng an einander liegende ungleiche rundliche Stücke (Fig. 75), deren der Oberfläche zunächst liegendes aus dem Ei gepresst wird."[1] (pp. 116, 117.) The figures show clearly the convexity of the curved nucleus directed toward the convex ventral surface of the egg, the gradual approximation of the nucleus to that surface, and the formation there of the polar cell, as described in the above passage. In the "Erklärung der Abbildungen," these figures (73–77) are said to be "Rechte Seitenansichten," which would make the convex surface and the place of polar-cell formation *dorsal*, as required by Zelinka's general statement. But this contradicts the description just quoted, and there is other proof that the orientation

[1] The Italics are mine.

of this first row of figures (73–80) of Melicerta is incorrectly given in the "Erklärung der Abbildungen." Thus, Figure 78 is said to be a "Dorsalansicht," which would bring the blastomere II ($= C^3$) upon the left side. But this blastomere is, in all other rotifers whose development is known, formed on the *right* side, and Zelinka states concerning this very figure, "Zuerst folgt der Kerntheilung die Zelltheilung in dem grossen Blastomer, die Theilungsebene steht senkrecht zur Kernspindel und schneidet ein Stück an der *rechten* Seite schief heraus (Fig. 78, II)."[1] (p. 117.) Again, Figure 79 is said to be a ventral view, whereas the same considerations as in the last case show it to be a *dorsal* view. Figure 80 is said to be a ventral view of the stage shown in Figure 78. Yet it is evidently a later stage than Figure 78; it really represents a ventral view of a stage similar to Figure 79, though the latter itself is said in the "Erklärung" to be a ventral view. In the text, Zelinka states correctly that Figure 79 is a dorsal, Figure 80 a ventral view (p. 118) of the same stage (p. 117), contrary to the statements in the "Erklärung der Abbildungen."

In Melicerta, therefore, the polar cell is formed in the same position as in Asplanchna Herrickii and Asplanchna priodonta, and marks the animal pole of the egg.

It seems very improbable that between these three forms and Callidina there should actually be such a difference in regard to the place of polar-cell formation as is brought out by the above comparisons. Zelinka's account of Callidina is full and clear upon this point; in both his description and his figures the polar cell is traced to a late stage, when a mistake in the orientation is impossible. There seems, however, to be one opportunity for error. The polar cell in Callidina is not embedded between the blastomeres, as in Asplanchna, but lies free upon the surface of the egg. This is shown by Zelinka's figures as well as by his descriptions. On page 62 he says, "Das Richtungskörperchen hat seinen Platz, den es früher eingenommen, verlassen und liegt nun ganz auf den kleinen Zellen." The egg of Callidina is enclosed by a rigid shell or membrane, which is separated from the egg itself by a space, except at the sides. The shell retains constantly the ellipsoidal form, while the egg itself may change its form and rotate within the shell. The original place of polar-cell formation, with respect to this shell, is near one end, — the end next to which *lies later* the anterior extremity of the embryo. But immediately after the first cleavage the egg rotates within the shell through an arc of about 90 degrees, and the region of polar-cell formation is carried to the equator of the egg. The polar cell itself is shifted at the same time, and it seems possible that during this rapid rotation it may be transferred to a region of the egg different from its original position. Such shiftings of the polar cell, though to a less extent, are mentioned by Zelinka (pp. 55, 58). During the "sehr schnell verlaufendes Phänomen" of the rotation of the egg within its shell, the relatively different shifting of the polar cell might have been overlooked. This is, however, only the suggestion of a possibility, for which there is no direct evidence in Zelinka's work.

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[1] The Italics are mine.

It is to be noted that in the above discussion I have employed throughout the orientation of the egg used by Zelinka, and not that adopted in my own account of the development.

B. Cleavage.

The first cleavage in the two species of Asplanchna differs from that of Callidina russeola in being exactly transverse to the long axis of the egg. In the latter form, according to Zelinka, the first cleavage plane is oblique to the spindle, and the spindle itself is oblique to the long axis of the egg. By a change of their relative positions immediately after division, the two cells are later brought into the same position relative to each other as in Asplanchna, and even at first the cleavage plane in the two forms occupies the same position *relative to the place of polar-cell formation.* The difference thus is of little importance, except that, from a cyto-mechanical standpoint, it shows that the form of the egg does not determine the position of the first cleavage spindle. In Eosphora also (Tessin, '86) the first cleavage plane is oblique to the long axis of the egg, whereas in Melicerta ringens (Zelinka, '91) and Asplanchna Sieboldii (Lameere, '90) the first cleavage plane is transverse to the long axis, as in Asplanchna Herrickii and Asplanchna priodonta.

The second and third cleavages in the two species of Asplanchna (Plate **1**, Fig. 6, Plate **2**, Figs. 8–16) are essentially similar to the corresponding cleavages of Callidina and of other rotifers in which the development has been described. For convenience in comparing the later stages, I give here a table showing the correspondence between the cells of Asplanchna in the eight-cell stage and those of Callidina.

Asplanchna.	Callidina (Zelinka, '91).	Asplanchna.	Callidina (Zelinka, '91).
$a^{4.1}$	a_1	$c^{4.1}$	II_1
$a^{4.2}$	a_2	$c^{4.2}$	II_2
$b^{4.1}$	b_1	$d^{4.1}$	I
$b^{4.2}$	b_2	$d^{4.2}$	III

Lameere ('90) and Leydig ('54), have given figures of the four-cell stage of Asplanchna Sieboldii. Beyond this stage there are no other published figures of the cleavage of any species of Asplanchna.

In regard to the fourth cleavage (Figs. 19–30, Plates **3** and **4**), a remarkable difference is to be observed between the cleavage of Asplanchna and that of Callidina as described by Zelinka. In Asplanchna the cleavage takes place up to (and beyond) this point with the greatest regularity both as to direction of spindles and as to sequence. The first and second cleavages are meridional, the third equatorial, and the fourth again equatorial. The sequence of cleavage is in every case D, C, B, A (see nomenclature of cleavage, page 16). In Callidina, according to Zelinka, the rhythm and regularity of the process is destroyed after the third cleavage by the remarkable circumstance that the cell $d^{4.1}$ (I, Zelinka) divides twice in succession before the fourth cleavage of any of the other cells. After these two divisions of $d^{4.1}$, the six cells of the other three quadrants are said to divide in the same succession that occurs in Asplanchna, while it is not until all these cleavages are finished that the cell $d^{4.2}$ (III, Zelinka) is separated into two blastomeres. Before this division of $d^{4.2}$ takes place, the egg consists in Callidina, as in Asplanchna, of four rows of four cells each, but in Callidina the method of origin of the four cells of quadrant D is stated to be different from that in the other quadrants. In this quadrant the three dorsal cells (posterior, Zelinka) are said to arise by successive cleavages of the large ventral (anterior, Zelinka) cell, while in each of the other three quadrants the four cells arise by the halving of the two cells previously present.

In Melicerta, according to Zelinka, the cleavage up to this point is as in Asplanchna; the cell I ($d^{4.1}$) divides first, then III ($d^{4.2}$), then the cells of the other three quadrants. Later the cleavage of Melicerta differs from that of Asplanchna, but up to the end of the sixteen-cell stage the processes are the same in the two.

In Eosphora, as described by Tessin ('86), the cleavage is like that of Asplanchna, except in the unessential particular that his cell a'' ($= d^{4.2}$) divides before the cleavage of a ($= d^{4.1}$). The sixteen-cell stage is reached by the cleavage of the same cells as in the two species of Asplanchna and in Melicerta.

In view of the regularity of the cleavage in these four forms, one might be led to suppose that the irregularity described in Callidina by Zelinka was due to defective observation. Zelinka has noted this point with particular attention, and states that he is certain of the difference between Callidina and Eosphora, as described by Tessin. Nevertheless it is possible that the slight *time* variation in the cleavage of Eosphora may have misled Zelinka as to the point which needed especial care. In Asplanchna Herrickii and Asplanchna priodonta, in Melicerta and in Callidina, the large cell $d^{4.1}$ divides first, followed immediately (in all except Callidina, at least,) by the division of $d^{4.2}$. In Eosphora, by a slight relative delay of the cleavage in $d^{4.1}$ (*a*, Tessin), the cell $d^{4.2}$ (*a″*, Tessin) divides first. Zelinka states that he has observed with especial care that the first cell (IV, Zelinka) given off in this quadrant takes origin from $d^{4.1}$ (I, Zelinka). This is doubtless true; the important point, however, is the origin of the *next* cell formed. Though this also is stated to arise from the ventral cell of the series (I, Zelinka), it seems possible that Zelinka was thrown off his guard by the supposed greater care necessary for determining the exact method of the preceding cleavage, and that the statement with regard to this one is really a mistake. The cell IV in Figure 23 (Taf. II.) of Zelinka's work might be the same cell as V in his Figure 24, while the cells called III and IV in Figure 24 might have arisen by the division of the previously existing cell III ($= d^{4.2}$). This would bring the conditions in Callidina into agreement with those in Eosphora, Melicerta, and the two species of Asplanchna. This is, of course, a mere suggestion, which indeed is rendered rather improbable by the nuclear conditions in the cells under discussion shown in Zelinka's Figure 24. There can, of course, be no question about the manner of division in Asplanchna. Figure 16 (Plate **2**) shows the spindles in $d^{4.1}$ and $d^{4.2}$, and the accomplished division of $d^{4.1}$ into $d^{5.1}$ and $d^{5.2}$ is shown in Figure 19 (Plate **3**), while $d^{4.2}$ still contains a spindle. I have observed similar conditions in many other specimens.

In view of the essential similarity of the process in Eosphora, as described by Tessin, to that in Melicerta, as described by Zelinka, and in the two species of Asplanchna, as observed by me, and in view of the fact that the cleavage of the quadrant in question (*D*) in these four forms may be said to agree completely with the general plan of cleavage as exhibited in the other three quadrants, — while in Callidina the conditions in this quadrant are anomalous, — the following remark of Zelinka ('91, p. 61) seems hardly justifiable: "Da, wie später gezeigt wird, auch Melicerta in der Entwicklung unserer vorliegenden Form folgt, so muss der Vorgang bei Eosphora als eine bemerkenswerthe Verschiedenheit aufgefasst werden." As above shown, the difference between Eosphora and the other forms consists merely in a slight variation as to the relative time of cleavage of two blastomeres ($d^{4.1}$ and $d^{4.2}$), — a phenomenon which is exceedingly common both in different individuals of the same species and in closely related species, and to which little or no significance can be attached. It is Callidina which shows a "bemerkenswerthe Verschiedenheit," since here the rhythm and regularity of the cleavage are completely destroyed, if the division is correctly described by Zelinka.[1]

Beyond the fourth cleavage it becomes very difficult to compare the processes in Asplanchna with those described by other observers for other rotifers. As above described, even in the fourth cleavage one of the cells of the *D* quadrant ($d^{5.3}$) was formed in a different manner in Callidina, according to Zelinka, from the method in Asplanchna. This in itself makes an exact comparison of the fifth cleavage in the two species impossible. But, considering for convenience the cells corresponding in position at the sixteen-cell stage as equivalent in the two forms, we find the following to be the process in Callidina as described by Zelinka.

The first blastomere of the sixteen-cell stage to divide in Callidina is said to be the dorsal cell of the *D* quadrant, $d^{5.4}$ (III, Zelinka), whereas in Asplanchna the very unequal division of the ventral cell $d^{5.1}$ takes place first (Fig. 33, Plate **4**, and Figs. 35 and 38, Plate **5**). The cleavage of $d^{5.4}$ is followed in Callidina by the division of $d^{5.3}$ and $d^{5.2}$ (IV and V., Zelinka). The division is meridional, as in Asplanchna (Fig. 37), but the products are equal, whereas in Asplanchna they are unequal.

Now, according to Zelinka, the products (at the fifth cleavage) of the division of $d^{5.4}$ ($d^{6.7}$ and $d^{6.8}$, Zelinka's III_1 and III_2) are themselves divided by meridional furrows. Thus the sixth cleavage takes place in these cells before the fifth has been accomplished in any of the other quadrants.

Following this, the large ventral cell of the *D* quadrant, $d^{5.1}$ (I, Zelinka), divides equatorially, giving off on its dorsal side a small cell, VI, which lies between the products of the division of $d^{5.2}$.

Thus the cleavage of the quadrant *D* is much less regular than in Asplanchna, where the ventral cells all cleave meridionally and unequally, the dorsal cell equatorially and unequally, the direction of cleavage being the same as in the other three quadrants (see Figs. 33 and 37). In Asplanchna, moreover, the fifth cleavage in the other quadrants is well advanced before any of the divisions for the sixth cleavage in quadrant *D* have taken place.

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[1] Zelinka's statement, quoted above, that Melicerta follows the same method of division as Callidina, depends merely upon his interpretation of a real variation; the actual divisions to form the sixteen-cell stage are the same in Melicerta as in Asplanchna and Eosphora, and different from those of Callidina, as may be seen by consulting Zelinka's ('91, p. 121) description.

Zelinka does not follow the cleavage in the other three quadrants cell by cell. He states that the dorsal cells of these quadrants (all?) are divided by planes parallel to the long axis of the egg, as is the case for all but the fourth or most dorsal layer in Asplanchna, and that the ventral cells $a^{5.1}$, $b^{5.1}$, and $c^{5.1}$ (a_1, b_1, and II, Zelinka) are the last to divide. In Asplanchna, as shown in Figures 39–44 (Plates **5** and **6**), all the cells of these quadrants divide meridionally except those of the dorsal layer, which divide equatorially.

It is obviously impossible to compare in detail the cleavage in the two forms at this time, or to reduce the condition described for Callidina to the regular scheme of cleavage exhibited in Asplanchna. Certain facts are perhaps worthy of notice, as showing the possibility that the cleavage in Callidina is not so different from that of Asplanchna as would be inferred from what is indicated above. The relation of the cells in quadrant *D* are somewhat similar in Figures 30 and 34 of Zelinka's work to a later condition in Asplanchna, — a condition reached, however, in a very different way, and shown in Figures 58 (Plate **7**) and 66 (Plate **8**). Further, the unequal cleavages of this quadrant are very confusing, and easily overlooked. Zelinka's work was apparently done almost entirely on living material, which does not lend itself as well as does preserved material to precise orientation of the object, and to its rotation in such a way as to permit views from all directions. It was only by bringing together a complete series, in which the karyokinetic process in every cell was represented in various stages by several specimens, that I was able to determine absolutely the course of events here. The remarkable unequal division of the cell $d^{5.1}$ is especially liable to be overlooked; I did not observe it till the break in the rhythm of cleavage at this point set me at work upon a minute study of a series of eggs separated in cleavage conditions by very short intervals only. It is worth noting that in Callidina, shortly after the time for this cleavage to occur, Zelinka figures (Taf. II. Fig. 31) a small vesicle lying almost exactly in the place occupied by the minute vesicle given off at this cleavage in Asplanchna, viz. between the ventral cells of quadrants *A* and *B* (a_1 and b_1, Zelinka). Zelinka considers this to be the polar cell, although at a previous stage he had observed that the polar cell had become displaced and now lay farther dorsad, on the outer surface of the smaller cells of the egg. The condition shown in his Figure 31 he ('91, p. 62) considers to be an exception. Later, he states that the polar cell becomes surrounded by small spherules, indicating that it is degenerating and falling to pieces. As shown in my Figures 51 and 52 (Plate **6**), the vesicle $d^{6.2}$ produced by the division of $d^{5.1}$ in Asplanchna also becomes surrounded by large granules or spherules; but these are not of the nature assumed by Zelinka; they are the result of a concentration of granules in the ventral cell $d^{6.1}$, traceable from the eight-cell stage onward. In view of these facts, it seems possible that a similar division actually takes place in Callidina, and that the small cell lying between a_1 and b_1 in Zelinka's Figure 31 is the small product of this cleavage, — the equivalent of $d^{6.2}$ in Asplanchna.

Tessin, in his study of Eosphora, also failed to follow the cleavage in detail to the 32-cell stage. He ('86, p. 282) speaks of "fortgesetzte Aequatorialtheilungen" of the cells in the three smaller quadrants; his figures show the three quadrants composed each of a single row of six cells (Figs. 22 and 23). This corresponds to the condition in Asplanchna at a time when the dorsal cells ($a^{5.4}-c^{5.4}$) have divided equatorially, but when the remainder of these three quadrants are as yet undivided. Next, all the cells, except the large ventral cell $d^{5.1}$, are said to divide meridionally. It is probable, therefore, that the formation of the minute cell by the division of $d^{5.1}$ was overlooked, and that the cleavage is essentially as in Asplanchna. The cells of quadrant *D* are said to divide unequally at this cleavage, as is also the case in Asplanchna.

The sixth and later cleavages of the ectoderm have not been studied in detail by other observers, so that a comparison of my results with observations on other forms is impossible. Diagrams of the sixth, seventh, and eighth cleavages for Asplanchna are given on pages 41, 46, 48, and 53, that for the eighth cleavage being incomplete.

The divisions of the entoderm cells have been followed somewhat further by Tessin and Zelinka, so that for these a comparison may be made.

Nothing comparable to the unequal fifth and sixth cleavages of the entoderm cell (forming the small vesicles $d^{6.2}$ and $d^{7.2}$), shown in Figures 38 (Plate **5**) and 49 (Plate **6**), have been reported by other observers.

Later than these the cleavage of the entoderm in Eosphora (Tessin, '86) takes place as in Asplanchna (Figs. 64, 65, and 76–83, Plates **8**–**10**) up to a stage comparable with that shown in Figures 77 and 78 (Plate **9**), except that no cleavage of the smaller dorsal cell $d^{8.2}$ corresponding to the unequal division which I have shown in Figure 80 (Plate **10**) was observed by Tessin. He did not follow the divisions of the entoderm further.

In Callidina a division takes place in the same manner as the seventh in Asplanchna (Figs. 64 and 65, Plate **8**), separating $d^{8.1}$ and $d^{8.2}$. The cleavage of $d^{8.1}$ into $d^{9.1}$ and $d^{9.2}$ also follows, as described above for Asplanchna.

An unequal cleavage of $d^{8.2}$, as shown in Fig. 80 (Plate **10**), was not observed in Callidina. The cleavages which next ensue are described by Zelinka as variable. The two cells corresponding to my $d^{9.1}$ and $d^{9.2}$ divide in the same direction as the corresponding cleavages of Asplanchna (Fig. 76, Plate **9**, and Fig. 81, Plate **10**), but the dorsal cells $d^{10.2}$ and $d^{10.4}$ are smaller than the others. The cell $d^{9.4}$ (*e*, Zelinka) divides by two successive divisions, at right angles to each other, into four cells; one of these divisions corresponds to that indicated for this cell in Fig. 83 (Plate **10**), while the other is at right angles to this. The order in which these cleavages occur in Callidina is, however, variable.

According to Zelinka, each of the four cells corresponding to my $d^{9.1}$, $d^{9.2}$, $d^{9.3}$, and $d^{9.4}$ now divides into three parts, but the details of these cleavages are not given.

In Melicerta the cleavage of the entoderm is traced by Zelinka to a four-cell condition, but the process is entirely different from that in Asplanchna, Eosphora, and Callidina, so that it would not be of interest to review the facts here.

The process of gastrulation takes place in Callidina and Eosphora, and probably in all other Rotifera, in a manner essentially similar to that in Asplanchna; the large ventral cell of the left posterior quadrant is enveloped by the other cells during the process of cleavage, and becomes the entoderm.

C. Summary on Maturation and Cleavage in the Rotifera.

In general, the following facts are shown for the early development of *Asplanchna*, as compared with previous accounts of the development of Rotifera.

1. The polar cell is formed at the animal pole of the egg, at a point opposite that where the blastopore is later found, and not at the dorsal or anterior margin of the blastoporic region, as stated by Zelinka for Callidina and Melicerta.

2. A much greater regularity, and in a certain sense symmetry, are shown in the direction and rate of cleavage than has been shown for

other Rotifera. Cell lineage is traced to a much later stage than has been done for other rotifers.

3. In other respects the development of Asplanchna, so far as observed, agrees in general with that of Callidina as described by Zelinka ('91). The development of organs was not traced in Asplanchna, the purpose of the work lying chiefly in the domain of cyto-mechanics.

PART THIRD. — MATERIAL AND METHODS.

The material for the studies here presented was collected by means of towings from Lake Michigan and certain small lakes connected with it, in August and September of 1894. Such towings were killed and preserved by a variety of methods. For killing, the following reagents were tried: (1) Flemming's stronger chrom-osmo-acetic mixture; (2) Kleinenberg's picro-sulphuric mixture, weaker solution; (3) Henneguy's fluid, consisting of Kleinenberg's weaker fluid plus 10% glacial acetic acid; (4) picro-nitric acid; (5) alcoholic corrosive sublimate; and (6) a mixture of corrosive sublimate and formalin. The best results were gained by the use of Flemming's mixture. The eggs were considerably darkened, but this defect was easily corrected by bleaching with chlorine generated from chlorate of potash and HCl. Henneguy's fluid and picro-nitric acid also gave good results. By alcoholic corrosive sublimate the eggs were commonly shrunk, and with Kleinenberg's fluid the shrinking was excessive. The towings after killing were preserved partly in 80% alcohol, and partly in a mixture of equal parts of glycerine, alcohol, and water. Both these preservatives gave satisfactory results.

As is well known, the development of the embryo takes place in Asplanchna within the body of the adult. The developing egg lies in the posterior part of the body of the mother, enclosed in the greatly distended oviduct or uterus, and with the ovary of the adult closely applied to it. It was necessary to pick out the Asplanchnas one by one from the quantities of Crustacea and other plankton with which they were mingled. This was done by using capillary tubes. It was necessary, moreover, to assort them with respect to the state of development of the contained embryo, if an embryo were present. This is a process involving great labor, as, in order to determine even approximately the stage of development of the embryo, it is necessary to examine the animal with the compound microscope. The majority of the specimens contain an embryo; not rarely two are present, in different stages of development, and in a single case I observed three.

In order to study the eggs, it is of course necessary to dissect them from the mother. This, again, is a tedious and delicate process, and it is rarely possible to free the egg entirely from the closely applied oviduct and ovary. This fact causes excessive trouble later, since the fragments of oviduct and ovary attached to the egg prevent one's placing it in any desired position or rolling it about at will, under a cover-glass.

For surface study of the early stages the eggs were then mounted in glycerine under a cover-glass supported by bits of capillary tubing thick enough to allow free motion to be given to the object. It was found impossible to use to advantage any stain for this study, because all the stains tried colored the cytoplasm more than the nucleus, and made the egg so opaque that cell boundaries and nuclei could not be distinguished. For the stages from one to about sixteen cells, eggs fixed in Flemming's solution are the best, as the slight darkening produced by this reagent is of advantage in stages where the egg is cleft into but few cells. For later stages this darkening is a disadvantage; eggs killed by other methods, or bleached after fixing with Flemming's fluid, must be used.

The eggs were moved about by rolling the cover-glass on its rollers, and drawings were made of different views thus obtained. It is here that the ovary and bits of oviduct attached to the egg cause infinite delay and vexation, in preventing the eggs from rolling easily or resting in any except certain positions. The time required for the work is certainly doubled, perhaps more than doubled, by this.

With early stages, camera drawings can be made at once from the egg after a favorable position is gained; but after the egg has reached a stage of about thirty cells, it is necessary first to roll the egg and make many tentative free-hand drawings of the different surfaces, until together they show the whole surface of the egg and the relation of every cell to all surrounding cells. The egg is then oriented and a camera figure made which shows the exact form of the cells in the middle region of the upper surface, and the position of *all the nuclei* of that surface. The cell boundaries about the periphery corresponding to these nuclei are then supplied from the free-hand sketches. This method, while not giving *mechanical* accuracy for the form of the cells about the periphery of a late stage of cleavage, does permit of complete accuracy so far as the relations of cells to one another are concerned; any other method with eggs in which the cell boundaries are so faintly marked on the surface is impracticable.

Sections were made of numbers of eggs, but optical sections are much more instructive, permitting exact orientation and revealing the structure fully as well as actual sections, so that most of the figures in which sections are represented were made from optical sections.

In the descriptive portion of this paper, details have been given of the movements of asters, nuclei, and other cell contents, as well as of the cells as units. As the entire account was gained from a study of preserved material, the question is a justifiable one, — Is there sufficient evidence that the movements actually occur as above described, or are the stages figured and described merely chosen at will from a mass of material and arranged arbitrarily in series?

The number of eggs used in determining the course of events in a given cleavage has been stated in several cases in the text. Thus, 31 eggs were studied containing more than one and less than five cells; 42 containing more than seven and less than sixteen, etc. In all, more than 250 eggs from Asplanchna Herrickii and 50 from Asplanchna priodonta, between the single cell stage (Fig. 1) and the stage containing five entoderm cells (Fig. 83), were mounted in glycerine and studied. Each egg, of course, came necessarily from a different individual, — since, where two embryos were present in the same adult, one at least had passed to a stage in the formation of organs. Of many of these eggs examinations were made which may be called exhaustive; i. e. every cell with its nuclear conditions was carefully *figured.* Thus, from the egg of which Figure 68 (Plate **8**) gives one view, at least twenty drawings were made, though but one is shown in the plates. The figures given, therefore, represent by no means even a considerable part of the evidence upon which the description is based. After determination of the exact order of events, drawings of typical cases were selected for illustrating the paper.

The determination of the sequence of the stages observed is greatly lightened by the almost entire constancy in the relative order of events in the different cells. Very slight variations occur in regard to certain processes, as in the case of the migration of the cloud of granules, as mentioned in the Explanation of Plates, under Figure 51. But, in general, a number of eggs representing a series of events in a given cell show corresponding series of events in the other cells. It is not necessary, therefore, to rely upon the conditions within the cell under examination for determination of the sequence of stages in this cell. Even this would probably be possible, however, from the fact that the nucleus in any cell increases in size steadily from the time the cell is formed to the beginning of the transformation of the nucleus and asters into the spindle figure — so that the relative size of the nucleus in the same cell from different eggs gives a measure of its relative age.

An illustration will make clear the method used. Perhaps the most difficult problem was presented by the movements of the asters and nuclei in the two large ventral cells of the quadrant *D*, at the fifth and sixth cleavages. These cells are $d^{5.1}$ and $d^{5.2}$ before the fifth cleavage; $d^{6.1}$ and $d^{6.3}$ *after* the fifth cleavage. Figure 31 (Plate **4**) shows the position of the asters in all the cells of this quadrant in the resting sixteen-cell stage. Figure 32 shows the nuclei in quadrant *B* in the same egg, as seen in a longitudinal section. In the egg shown in Figure 33 the spindles are already completed in quadrant *D*, indicating that this egg is older than that shown in Figures 31 and 32. Figure 35 (Plate **5**), a ventral view of this stage, shows that the nuclei in $a^{5.1}$ and $b^{5.1}$ are much larger than the nuclei in the corresponding cells of Figures 30 and 31, — which likewise indicates that this is an older stage than is represented in the latter figures. Figure 35 shows the exact antero-posterior position of the spindle in $d^{5.1}$, while in the other quadrants the nuclei are shown to be still in a resting condition.

Figures 37 and 38 together show all the cells of this quadrant during or just after division, the four dorsal cells being still connected by interzonal filaments. It is therefore a stage *later* than that shown in Figures 33 and 35. This is in agreement with the larger size of the nuclei in quadrant *A*, Figure 37, as compared with those of quadrant *A* in Figure 33, and also with the presence of a spindle in $a^{5.4}$. In this egg the nucleus in $d^{6.1}$ (Fig. 38) has resumed its nearly spherical form, and at the side of the cell where the anterior end of the spindle was located (Fig. 35) is a small vesicle, $d^{6.2}$ (Fig. 38). This represents one product of the division.

In $d^{6.3}$ at this stage (Figs. 37 and 38). the single aster is slightly extended in a direction which is oblique to both the longer and shorter axes of the cell.

The spindles in the cells of quadrants *A*, *B*, and *C* in Figures 39–42 (all from the same egg) prove that this is a later stage than that last considered. In this egg the line joining the two asters in $d^{6.1}$ (Fig. 42) is oblique to the antero-posterior plane of the embryo, and nearly dorso-ventral.

In the egg shown in Figures 43–46, division is completed in some of the cells of quadrants *A*, *B*, and *C*, proving this to be a later stage than those shown in Figures 37–42. In this egg the two asters in $d^{6.3}$ (Fig.

46) are at opposite sides of the nucleus, the line joining them being parallel to the lateral axis of the embryo.

In the egg seen in Figure 53 (Plate **7**), the fifth division is *finished* in all the cells of the egg, (as shown by the study of the other quadrants, which are not figured,) so that this is a later stage than that shown in Figures 43–46. The asters in $d^{6.3}$ have taken an oblique position.

In the egg of which Figures 48 (Plate **6**) and 54 (Plate **7**) are representations, the nuclei in the recently formed cells of quadrants A, B, and C have enlarged, and the spindle is completely formed in $d^{6.3}$ (being dorso-ventral in position). Both of these facts show that the egg is older than the one shown in Figure 53. In this egg (Fig. 48) we find that a spindle is present in the entoderm cell $d^{6.1}$, occupying nearly the position foreshadowed by the position of the asters in Figure 42, and almost exactly the same position as the spindle at the foregoing division (Fig. 35).

The egg seen in Figure 49 is still older, as shown by the presence of spindles in $d^{6.5}$ and $d^{6.7}$ (seen endwise), and the advanced condition of cleavage in $d^{6.8}$. Here $d^{6.1}$ is just dividing, forming $d^{7.1}$ and the second small cell, $d^{7.2}$.

Figure 50 is older than Figure 49, since $d^{6.5}$, $d^{6.7}$, and $d^{6.8}$ have divided. In this egg we find that $d^{6.8}$ has been separated into two cells, $d^{7.5}$ and $d^{7.6}$, and there is a second small vesicle, $d^{7.2}$, in the position where it was seen in the process of formation in Figure 49.

The above is sufficient to illustrate the method of work; the rest of the account might be analyzed in the same way. It is important to remember, however, that the description is not based merely upon the cases figured. Thus, for the processes just analyzed, more than thirty eggs, showing various phases of the changes occurring, were studied, while only eleven different eggs are represented in the figures of these stages.

The foregoing work was done in the winters of 1894–95 and 1895–96, in the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard University. It gives me pleasure to acknowledge my great indebtedness to the Director of the Laboratory, Professor E. L. Mark, for advice and assistance which have been of the greatest value to me throughout my work.

GENERAL SUMMARY.

A. Observations.

1. Many divisions take place during the cleavage of Asplanchna in which the spindle lies in the *shortest axis* of the cell, in the direction of *greatest pressure*, and the ensuing division results in the production of contact surfaces of *greatest area.*

2. In the cleavage of the ectoderm of Asplanchna any cell of any one quadrant cleaves in the same direction as the corresponding cell of the other quadrants, though the forms of the corresponding cells may vary excessively. Conversely, cells of the same form and with similar relations to surrounding cells, but belonging to different layers or series, may divide with spindles in exactly opposite directions.

3. The entodermal cell follows the same rhythm and direction of cleavage as the other cells, so long as it remains on the exterior and thus corresponds in position with other cells of the egg. When it becomes enveloped by the other cells, so as to come into different relations with the axis of the embryo, its plan of cleavage changes, showing no definite relation to that of the ectoderm.

4. All the cleavages in the ectoderm are to a late period either equatorial or meridional, so that the position of any given spindle is either parallel or perpendicular to that of the preceding spindle.

5. There is no regular alternation in the direction of spindles. Equatorial cleavages may follow successively for three or more generations, and the same is true of meridional cleavages.

6. The position occupied by the two asters after they have passed to opposite sides of the nucleus does not indicate the direction of the ensuing spindle. This may occupy the position indicated by the asters, or the definitive position may be gained by a rotation of the asters and nucleus at the passage into the karyokinetic condition.

7. There is no "regular angle of rotation" (Heidenhain) in a mechanical sense, since (a) in cells of different layers, in one case the angle may be zero, in the other case 90 degrees; and (b) even in cells where the direction of the previous spindle and the direction of the following spindle are the same, the asters may move in an entirely different manner. In one cell the rotation may be directly through an angle of 90 degrees, and in a single plane, while in another there may be complex movements and rotation successively in different planes.

8. The position and movements of the asters in the resting stage seem partly determined by the form of the cell.

9. The rotation of the nucleus and asters into the definitive position at the time of karyokinesis often takes place *from the longer into the shorter axis of the cell*, and apparently from the direction of least pressure into the direction of greatest pressure.

10. The form of the cells in many cases does not conform to the law of minimal surfaces, being (a) changeable, and (b) even in the resting stage widely at variance with the conditions required by the law.

11. Many of the cleavages are unequal, sometimes extremely so, but the inequality shows no significant relation to accumulations of yolk material. (See 16.)

12. The sequence of cleavage is (within very narrow limits of variation) constant, and shows no relation to accumulations of yolk. There is a general tendency for larger cells to divide faster, but not all the facts regarding the succession of cleavages show relation to the comparative size of the cells.

13. In the resting stage the cells seem to be passive, taking whatever form is impressed upon them by the surrounding cells. As the cell passes into the karyokinetic condition it becomes more rounded, the cytoplasm tends to group itself symmetrically about the spindle, and the cell elongates in the direction of the spindle.

14. The spindle generally (not always) lies in either the longest or the shortest axis of the cell, as maintained by Roux. But apparently this is due in Asplanchna to the fact that the cytoplasm tends to group itself symmetrically about the spindle.

15. A change of the relation of a cell to the axes of the egg, as by a displacement due to the other cells, results in a change of the position of the spindle with reference to the axes of the egg.

16. During cleavage a cloud of granules is segregated in a portion of the cell which is to form the entoderm; this mass passes from thé anterior and ventral side of the entoderm cell to its posterior and dorsal side, and is there separated off at the seventh cleavage into the *smaller* entodermal cell.

17. The egg retains its ellipsoidal form throughout all the processes of development, up to a late stage, though as cleavage progresses the blastomeres shift extensively their positions with relation to this form. This retention of the ellipsoidal form by the egg cannot be referred to any simple mechanical factor. (See pages 81, 82.)

18. Gastrulation accompanies cleavage, and advances step by step

with the withdrawal of the deep parts of the peripheral cells and their dorso-ventral extension, consequent upon frequent equatorial divisions.

19. As to facts bearing upon the special morphology of the Rotifera, —

(a) The polar cell is formed at the animal pole of the egg, at the point opposite that where the blastopore is later found, and not at the dorsal (or anterior) margin of the future blastoporic region, as stated by Zelinka ('91) for Callidina.

(b) The cleavage of Asplanchna was traced to a later stage than has been done for other rotifers. A much greater regularity, and in a certain sense symmetry, are shown in the direction and rate of cleavage than has been shown for other species.

B. Conclusions.

20. It results from 1, 2, 3, 5, 7, and 9 that the direction of cleavage is not determined by any simple mechanical factors or relations of form. Specifically, the course of cleavage in Asplanchna is inconsistent with any general validity of (1) Hertwig's law of the spindles in the longest axis of the protoplasmic mass, (2) Berthold's law of least areas, and (3) Braem's and Pflüger's principle of least resistance.

21. It results from 11 that no simple factor can account for the equality or inequality of the cleavage. Specifically, the conditions in Asplanchna are inconsistent (a) with Hertwig's view that the dividing nucleus takes a position "in the middle of its sphere of action," so far as that expression has any definite significance, and (b) with Braem's principle of "like resistance" at the two ends of the spindle.

22. It results fron 12, as well as from a comparison with the cleavage of many other invertebrates, that no simple factor, such as greater or less quantity of yolk, will account for the sequence of cleavage.

23. It is a natural conclusion from 15 and the latter part of 14, that the direction of the spindle is not due to an influence in the egg as a whole, connected with its axial relations, but is determined within each cell itself. However, I do not consider this conclusion at all well established.

24. It results from 5, 6, 7, 8, and 9 that the problem as to what determines the position of the spindle is resolvable into several: (a) What determines the direction of separation of the newly formed asters? (b) What determines the position of the asters during the resting stage

of the cytoplasm? (c) What determines the rotation of the asters and nucleus as the cell passes into the karyokinetic condition?

25. It may be concluded from 20, 21, 22, and 24 that the final position of the spindle and manner of cleavage are causally determined by processes — of an unknown character — taking place within the protoplasm.

26. The definite relation of the position of the spindle to external conditions observed in some cases — such as to the form of the cell, the direction of pressure (?), and the direction of the incoming rays of light — is to be interpreted as a reaction to stimulus, dependent in every case upon the specific structure of the protoplasm, and variable with that structure.

27. The manner of division is related to the purpose to be attained by the given division, and to the general morphogenetic changes in the organism. In Asplanchna the method of cleavage is adapted to bringing about gastrulation.

28. It follows from 16 that cleavage is not merely a quantitative division into similar units; it is accompanied by other developmental processes, some of which are distinctly traceable.

29. Gastrulation in Asplanchna is not a process distinct from cleavage, but is an accompaniment and a result of cleavage. The process of which it forms a part begins at the third cleavage and is not finished until much later than what is commonly spoken of as gastrulation proper.[1]

30. Gastrulation in Asplanchna may be analyzed into several factors.[1]

(a) The form of the egg, or the influences determining it.
(b) The direction of cleavage.
(c) The inequality of cleavage.
(d) The sequence of cleavage (?).
(e) The changes in form taking place as the cells divide.

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[1] It may be well to state expressly that I do not consider the above as in any sense a general explanation of the process of gastrulation. My aim has been to give as nearly as possible a correct account, from the standpoint of developmental mechanics alone, of the facts in regard to the early development of a single form. The origin of the process of gastrulation in phylogeny is not touched by this account. It is a common phenomenon in the organic world, that the same end is accomplished by different means in different cases; doubtless in many forms gastrulation is brought about in a way that bears no resemblance to the process in Asplanchna. In general, the whole question of the origin of processes to which an end or purpose can be assigned lies entirely without the field of the present paper.

All of these must, according to 17, 25, and 26, be considered as determined by the unknown (molecular?) structure and activities of the protoplasm.

31. It follows from 30 that the early development of Asplanchna, to a stage somewhat beyond gastrulation, may be analyzed into two factors: (1) the influences determining and preserving the form of the egg as a whole, and (2) processes occurring in consequence of the specific (molecular?) structure and activities of the protoplasm.

Both of these factors, which perhaps should be considered as different manifestations of one, are from a causal-mechanical standpoint, entirely unknown. "Damit werden die causalen Bedingungen der Entwicklung vorzugsweise in das Moleculargeschehen verlegt und entziehen sich vorderhand grossentheils unserer weiteren Erforschung." (Roux, '85^a, p. 427.)

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EXPLANATION OF PLATES.

All the figures represent preparations of the eggs of Asplanchna Herrickii de Guerne, except Figure 29, Plate **4**, which represents the egg of Asplanchna priodonta Gosse. All were drawn, with the aid of the Abbe camera lucida, to a magnification of 525 diameters.

The four blastomeres of the four-cell stage are distinguished by different colors, and the same color is retained throughout for all the cells (constituting a "quadrant") derived from each of the four blastomeres thus distinguished. The quadrant *A* is blue; *B*, orange; *C*, yellow; and *D*, red.

The prominent granulations in the ventral portion of quadrant *D* are figured; but all other granulations are omitted, except in the case of Figure 7, where the general granulation is also represented, though somewhat diagrammatically.

For an explanation of the system of nomenclature used in lettering the cells see page 16. In some cases for want of room the letters have been omitted, the exponents only being expressed. In such cases the color indicates to which quadrant the cell belongs.

In all the figures where it is possible, the animal pole of the egg is above, the ventral pole below. In views of the dorsal or ventral poles of the egg, the anterior end is above. Unless otherwise stated, figures represent surface views of more or less transparent eggs; these are shaded, whereas sections — optical or actual — are not shaded.

ABBREVIATIONS.

bl'po.	Blastopore.	*cl. pol.*	Polar cell.	*pol. anm.*	Animal pole.

PLATE 1.

Fig. 1. Egg showing the maturation spindle.

Fig. 2. Egg slightly older than that shown in Figure 1; the polar cell is formed and is embedded in the egg; the cleavage nucleus, preceded by the deep aster of the maturation spindle, is moving from the place of polar-cell formation toward the interior of the egg.

Fig. 3. First cleavage spindle, early stage.

Fig. 4. Longitudinal section (actual). The two nuclei formed at the first cleavage are separating; the notch and the granules at the periphery indicate the beginning of the formation of the first cleavage plane. The aster in the anterior (upper) end of the egg has divided, while the opposite aster is still entire.

Fig. 5. Two-cell stage seen from the dorsal side; the anterior end above. There are two asters in the cell $\overline{AB}^2$, while the aster in $\overline{CD}^2$ is still undivided.

Fig. 6. Two-cell stage, from the dorsal side; spindles in both blastomeres.

Fig. 7. View of an eight-cell stage; optical section through the quadrants B and D, showing the distribution of the yolk granules. Observe the concentration of granules in the ventral part of $d^{4\cdot 1}$, and the position of the asters in $d^{4\cdot 1}$ and $d^{4\cdot 2}$.

pol
Mb
Ch

PLATE 2.

Fig. 8. Four-cell stage, viewed as a transparent object from the animal pole. The anterior end is above.

Fig. 9. Four-cell stage, later than Figure 8, seen from the right side. Spindles in C^3 and D^3; spherical nucleus in B^3.

Fig. 10. Four-cell stage, optical section through the cells B^3 and D^3, showing the division of the asters in preparation for the third cleavage.

Fig. 11. Five-cell stage, from the ventral side. The anterior end is above. The aster in $d^{4\cdot1}$ has divided laterally, the two parts being still connected. Spindles in A^3, B^3, and C^3 seen endwise.

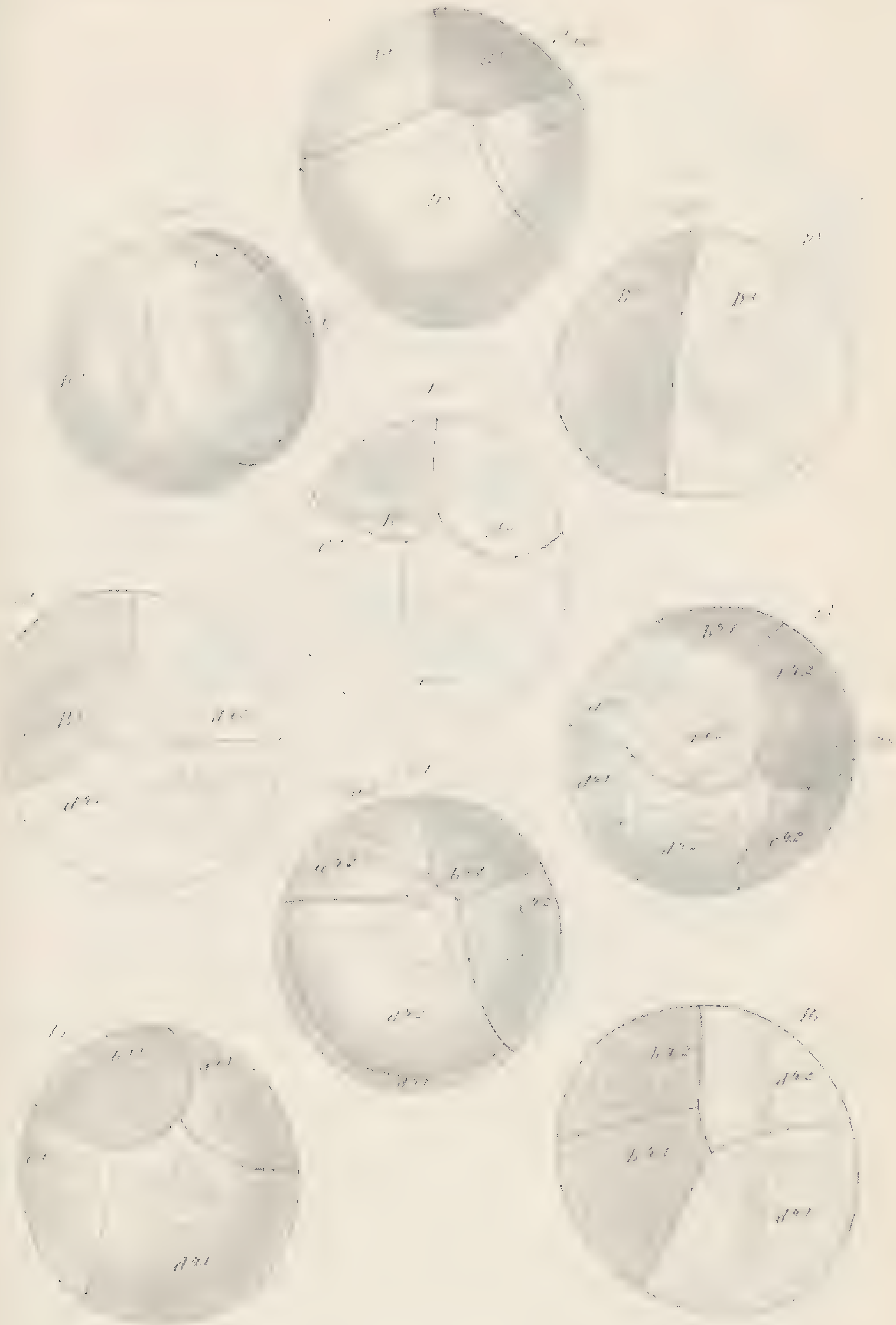
Fig. 12. Optical section, approximately sagittal, through the quadrants *B* and *D*, from the egg shown in Figure 11.

Fig. 13. Oblique view of the eight-cell stage, shortly after the third cleavage. The animal pole is marked by the polar cell; the anterior median line by the boundary between quadrants *A* and *B*.

Fig. 14. Dorsal view of the egg represented in Figure 13, showing the oblique position of the asters in $d^{4\cdot2}$, and the lateral extension of the aster in $c^{4\cdot2}$.

Fig. 15. Ventral view of the egg seen in Figures 13 and 14, showing the oblique position of the asters in $d^{4\cdot1}$, and the lateral extension of the aster in $c^{4\cdot1}$.

Fig. 16. Optical, nearly sagittal, section of the eight-cell stage, through the quadrants *B* and *D*, showing the dorso-ventral direction of the spindles in $d^{4\cdot1}$ and $d^{4\cdot2}$. Notice also the change of form of the quadrant *B*, as compared with the same quadrant in Figures 10 and 12.



B³
b⁴¹
c⁴²
d⁴¹
d⁴²
b⁴²
c⁴²
d⁴²
b⁴²
d⁴²
b⁴¹
d⁴¹
b⁴¹
d⁴¹
d⁴¹

PLATE 3.

Fig. 17. Right side of the eight-cell stage, same egg as that seen in Figures 13, 14, and 15, showing the lateral extension and beginning of division of the asters in $c^{4\cdot1}$ and $c^{4\cdot2}$.

Fig. 18. Right side of a slightly older stage than Figure 17, showing the completion of the division of the asters. In $c^{4\cdot1}$ the line joining the asters is lateral; in $c^{4\cdot2}$ it has already become oblique.

Fig. 19. Right side of a stage later than that shown in Figure 18, containing nine cells. The line joining the asters in $c^{4\cdot2}$ has become dorso-ventral, while that joining those of $c^{4\cdot1}$ has become oblique. The cleavage of $d^{4\cdot1}$ into $d^{5\cdot1}$ and $d^{5\cdot2}$ has occurred (compare Fig. 16), while $d^{4\cdot2}$ is still undivided.

Figs. 20–25. Different views of an egg in the ten-cell stage, to show the position of the spindles in relation to the exact form and dimensions of the cells.

Fig. 20. Right side of a stage later than Figure 19, but containing still only ten cells. The line joining the asters in $c^{4\cdot1}$ has become dorso-ventral and the spindle is formed between them. Likewise in $c^{4\cdot2}$; $d^{4\cdot2}$ has divided into $d^{5\cdot3}$ and $d^{5\cdot4}$.

Fig. 21. Anterior surface of the egg represented in Figure 20; the spindles occupy the shorter axes of $b^{4\cdot1}$ and $b^{4\cdot2}$.

Fig. 22. Left side of same egg showing the nuclear conditions in quadrant *A*. The spindles are not yet formed.

Fig. 23. Right face of optical, nearly sagittal, section, through quadrants *B* and *D*, from the egg shown in the three preceding figures, to exhibit the exact dorso-ventral extent of the cells $b^{4\cdot1}$ and $b^{4\cdot2}$, as compared with the lateral extent of the same cells in Figure 21.

Fig. 24. Dorso-ventral, approximately frontal, optical section of the egg shown in Figures 20–23, showing the greatest dorso-ventral extent of the cells of quadrants *A* and *C*, for comparison with the lateral dimensions of the same cells, shown in Figures 20 and 22.

Fig. 25. Posterior surface (quadrant *D*) of the egg shown in Figures 20–24.

PLATE 4.

Figs. 26–29. Ten-cell stage.

Fig. 26. Left side of an egg in a ten-cell stage, slightly older than that shown in Figures 20–25. Note the dorso-ventral elongation of the cells $a^{4.1}$ and $a^{4.2}$, as compared with the same cells in Figure 22.

Fig. 27. Right side of the egg shown in Figure 26. The cytoplasm is beginning to become constricted in the cells of quadrant *C*.

Fig. 28. Dorso-ventral, approximately frontal, optical section of the egg shown in the two preceding figures, viewed from the anterior side, to show the greatest dorso-ventral extent of the quadrants *A* and *C* at this stage.

Fig. 29. Right side of the egg of Asplanchna *priodonta* Gosse, in the ten-cell stage, showing the spindles in the shorter axes of the cells.

Figs. 30–36. Sixteen-cell stage.

Fig. 30. Resting condition, seen from the anterior side.

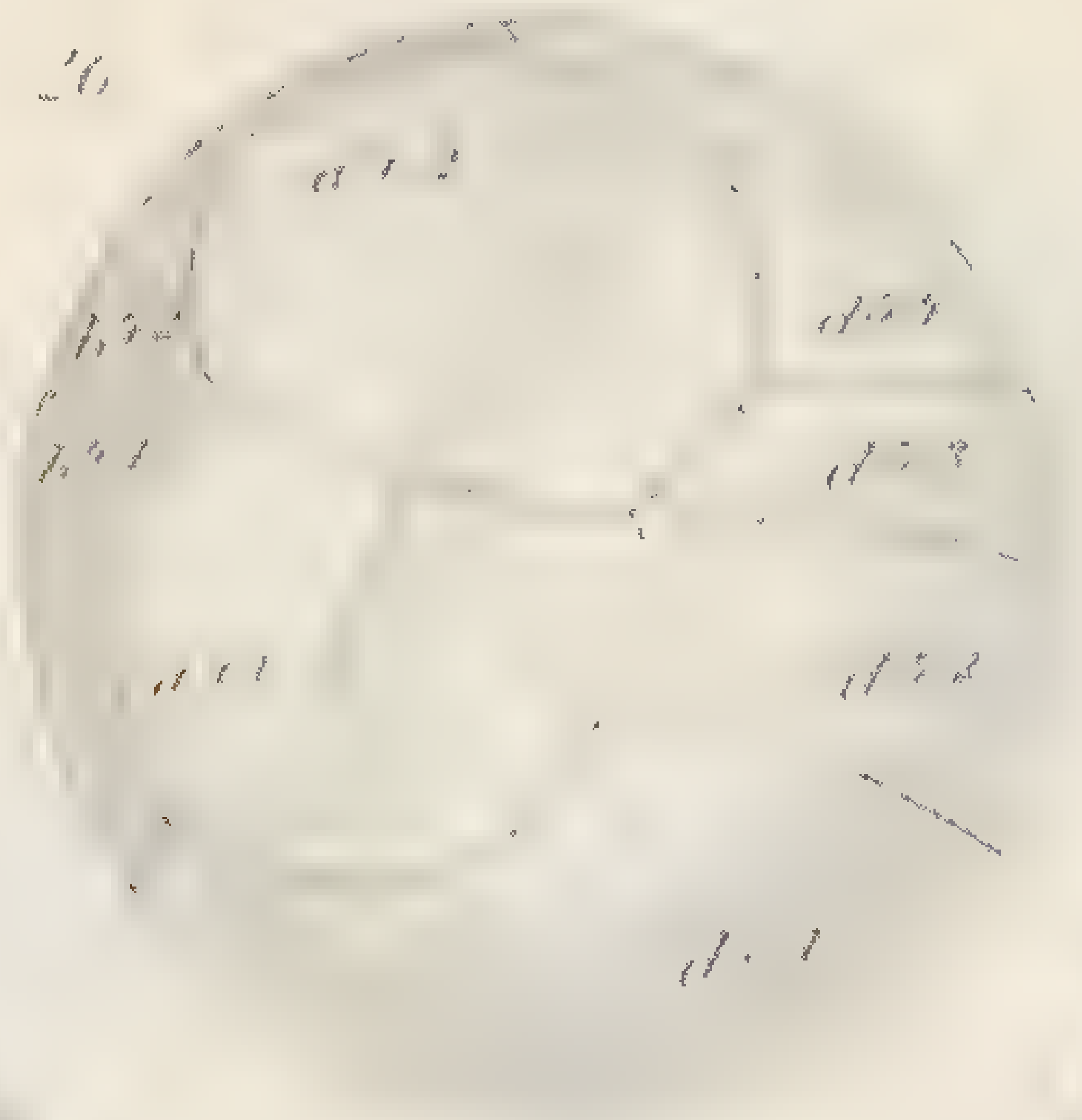
Fig. 31. Left posterior view of the egg seen in Figure 30, showing the position of the asters in all the cells of quadrant *D*.

Fig. 32. Dorso-ventral, nearly sagittal, optical section through the quadrants *B* and *D*, from the egg shown in the two preceding figures.

Fig. 33. Posterior view of an egg slightly older than that shown in Figures 30–32.

Fig. 34. Nearly sagittal optical section, through the quadrants *B* and *D*, in the same stage as that shown in Figure 33.

26



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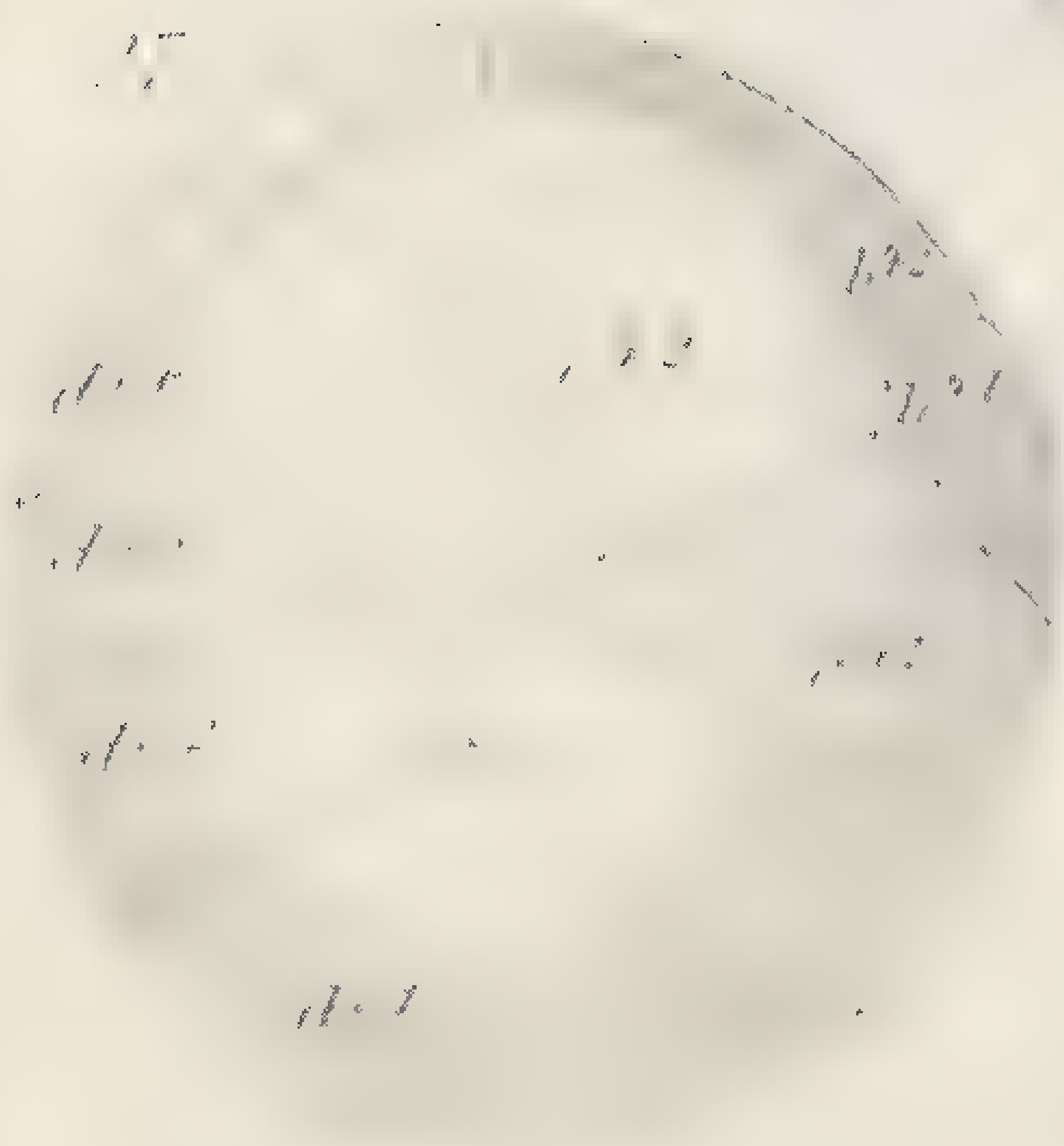

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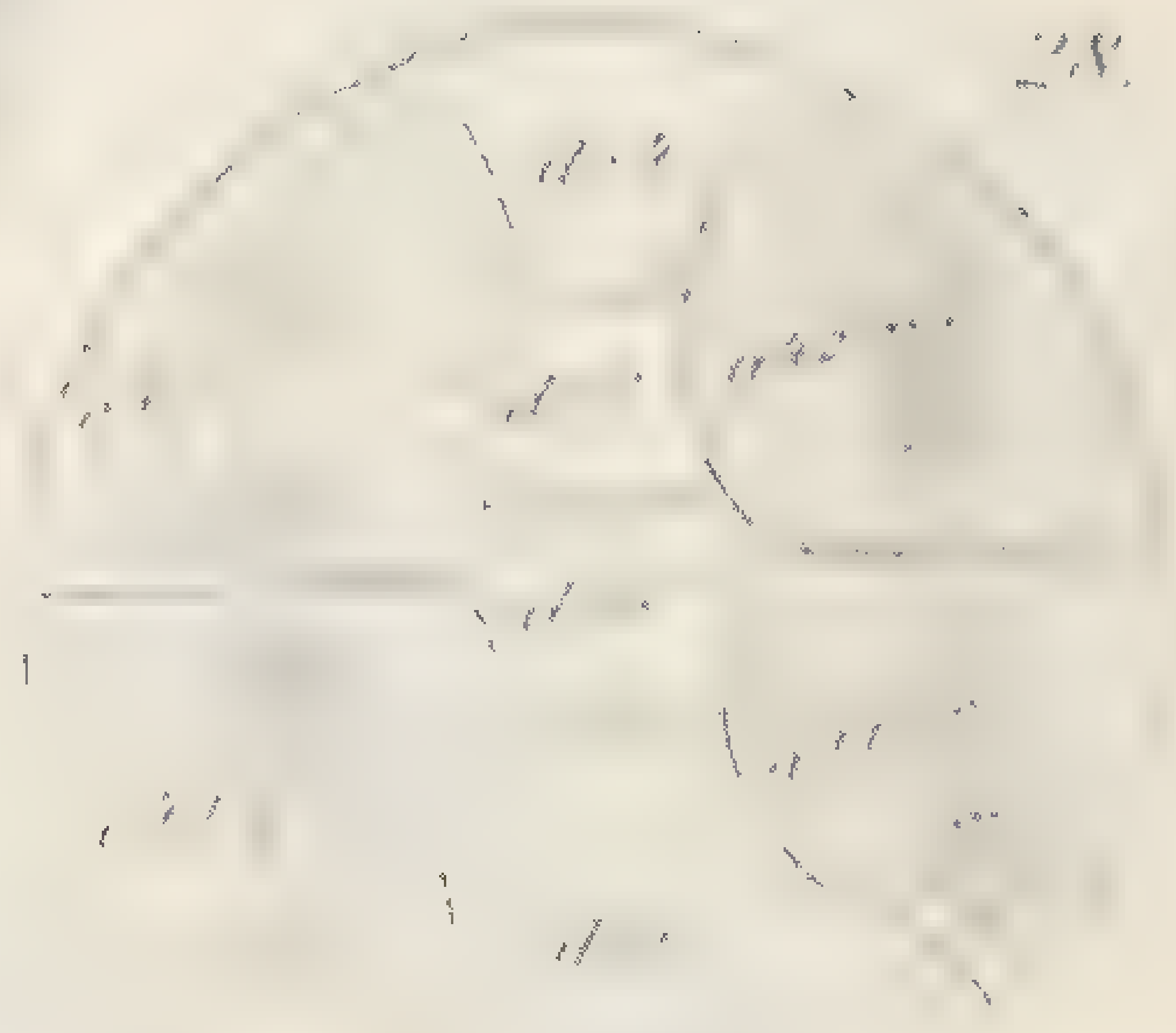

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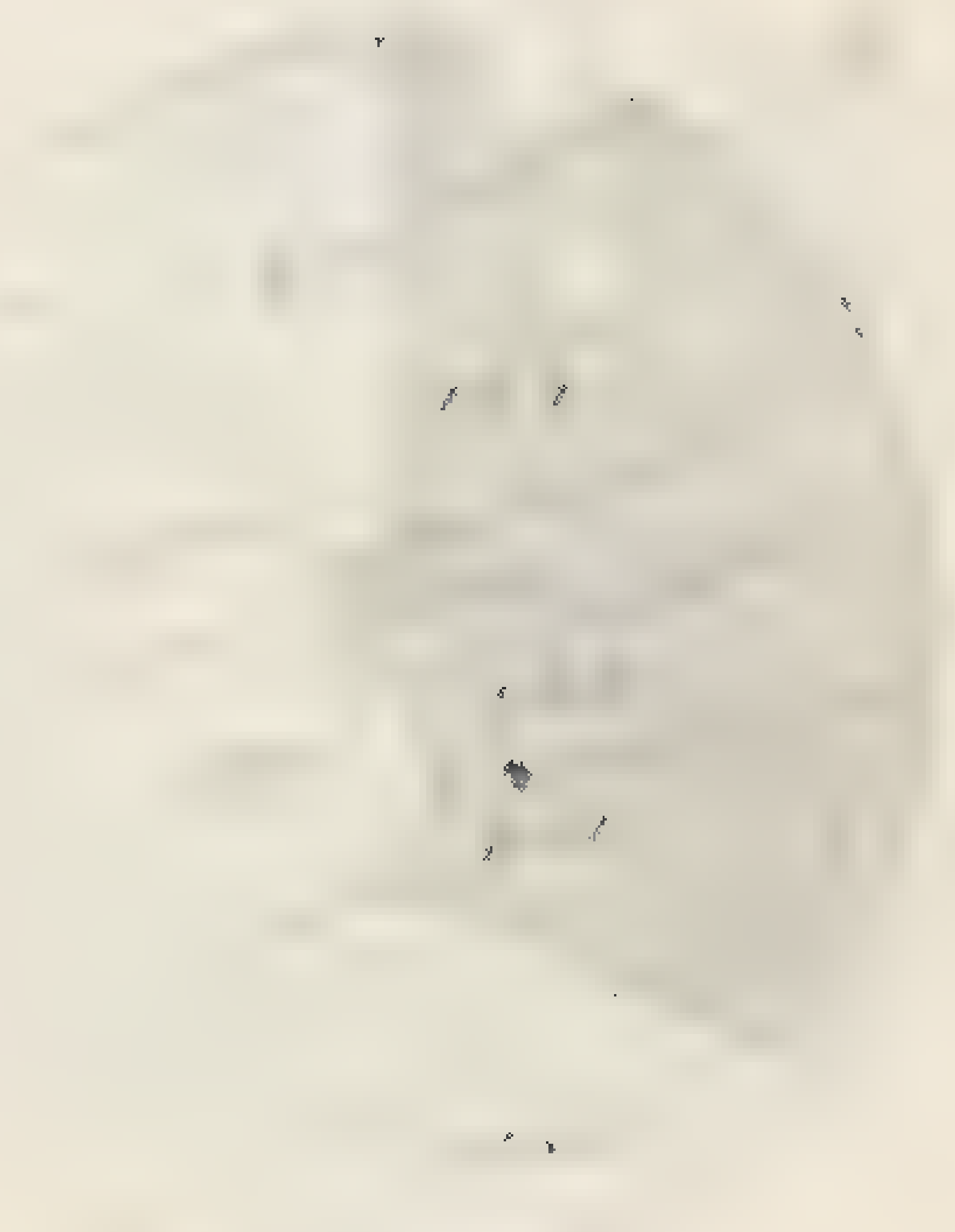


PLATE **5**.

Fig. 35. Ventral view of a stage similar to that seen in Figures 33 and 34, Plate **4**, showing the antero-posterior position of the spindle in $d^{5.1}$.

Fig. 36. Transverse optical section of the sixteen-cell stage, through the cells of the third layer, exhibiting the position of the spindle in $d^{5.3}$. The section is viewed from the dorsal side. (Compare Plate **2**, Fig. 8.)

Figs. 37–42. Twenty-cell stage.

Fig. 37. Completion of the fifth cleavage in quadrant *D*. Note the oblique position of the elongated aster in $d^{6.3}$.

Fig. 38. Sagittal optical section of the egg shown in Figure 37. The section passes, on the anterior side, between the cells of quadrants *A* and *B*, showing the cells of quadrant *A*.

Figs. 39–42. Different views of one and the same egg.

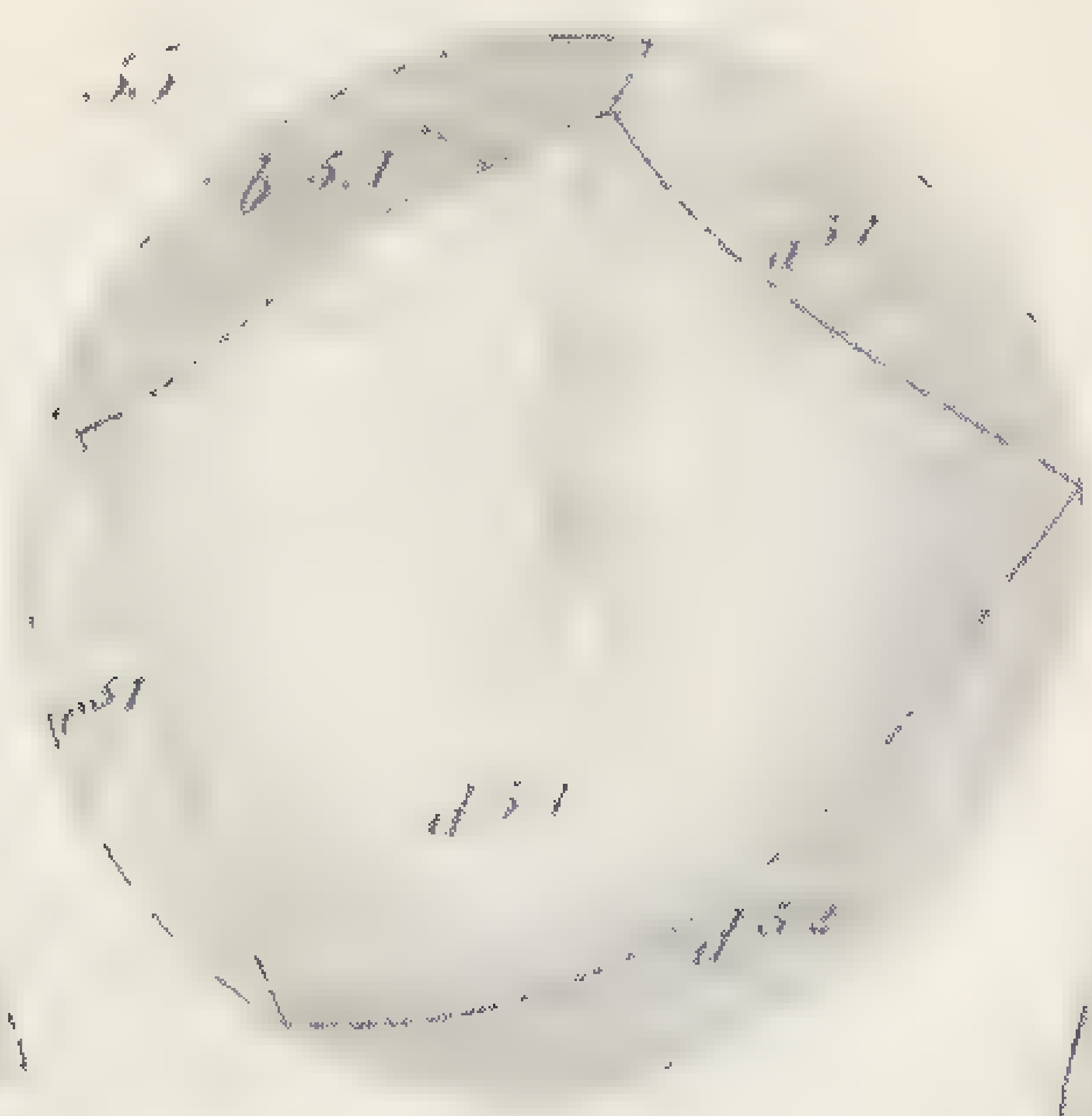
Fig. 39. Left side, showing the asters and spindles for the fifth cleavage in quadrants *A* and *B*.

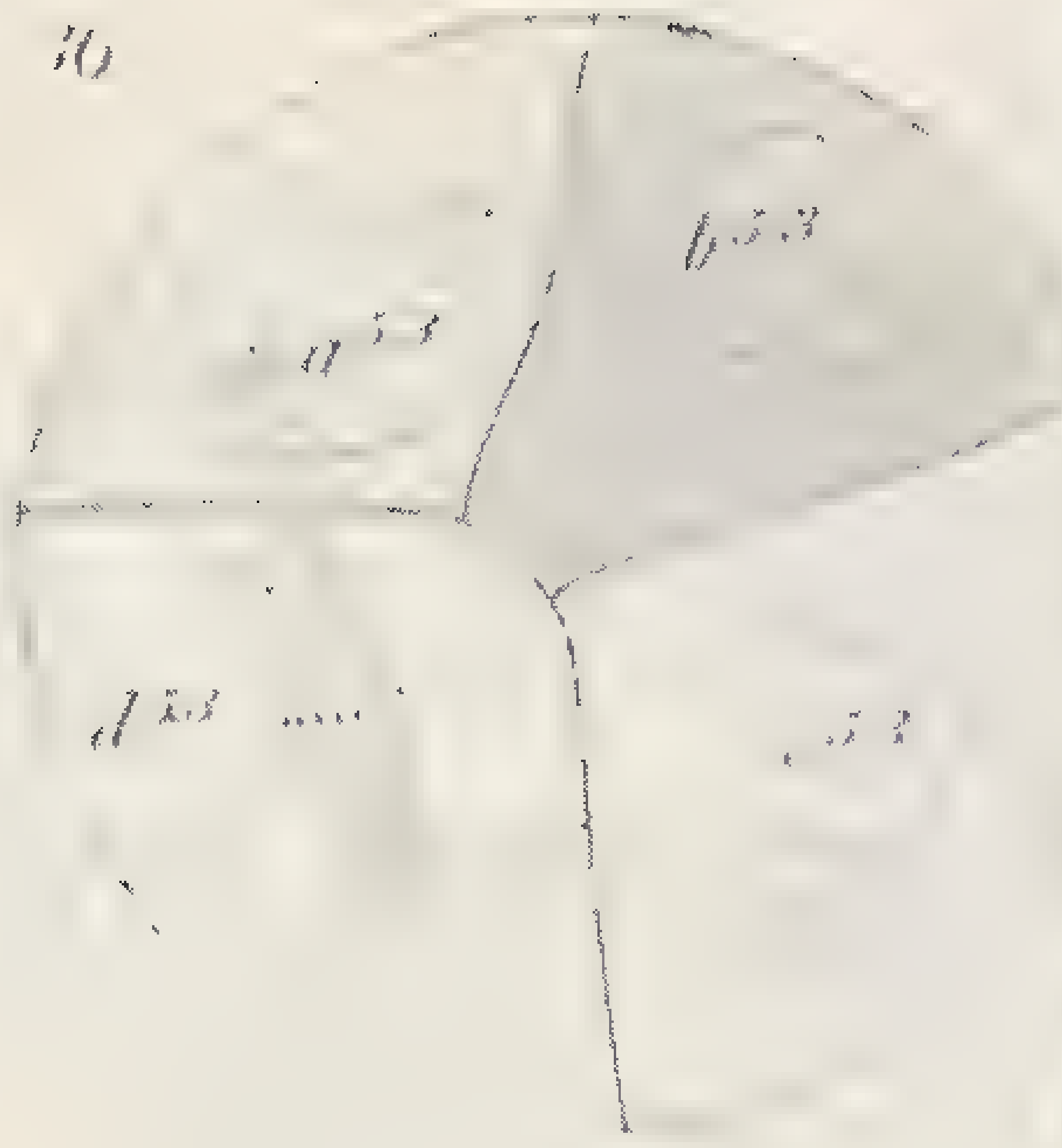
Fig. 40. Right side of the egg shown in Figure 39. Observe the more advanced karyokinetic stages in quadrant *C*, as compared with quadrant *A*, Figure 39.

Fig. 41. View looking down upon the animal pole.

Fig. 42. View of the same egg from the ventral pole, showing the oblique position of the asters in $d^{6.1}$.

Fig. 43. An older stage than that given in Figures 39–42; the fifth cleavage in progress; the egg contains twenty-seven cells. The cells $c^{5.3}$ and $b^{5.3}$ are forced apart by the cell $c^{6.7}$.


55
b 5.1
a 51
b 51
d 51
d 52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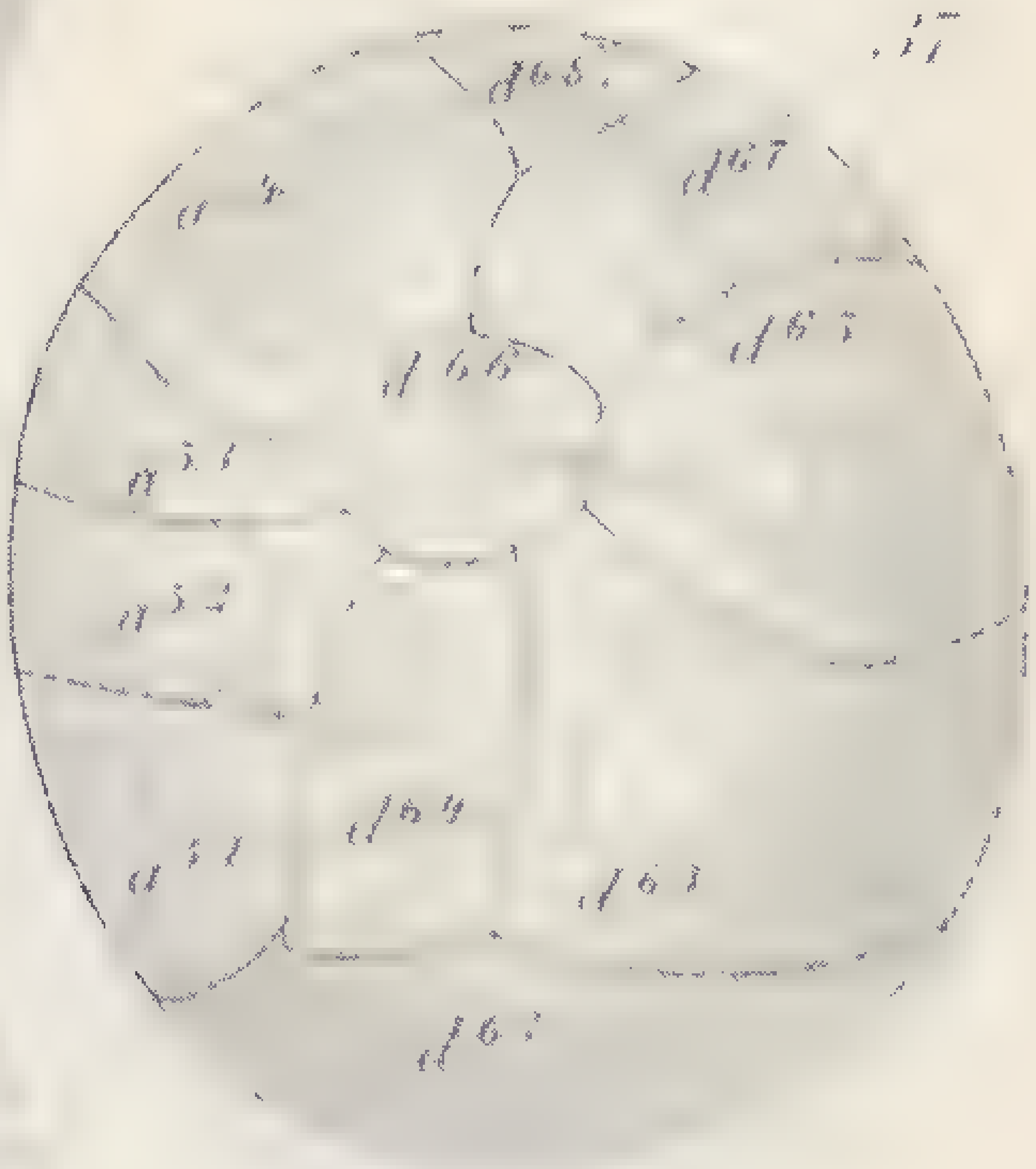
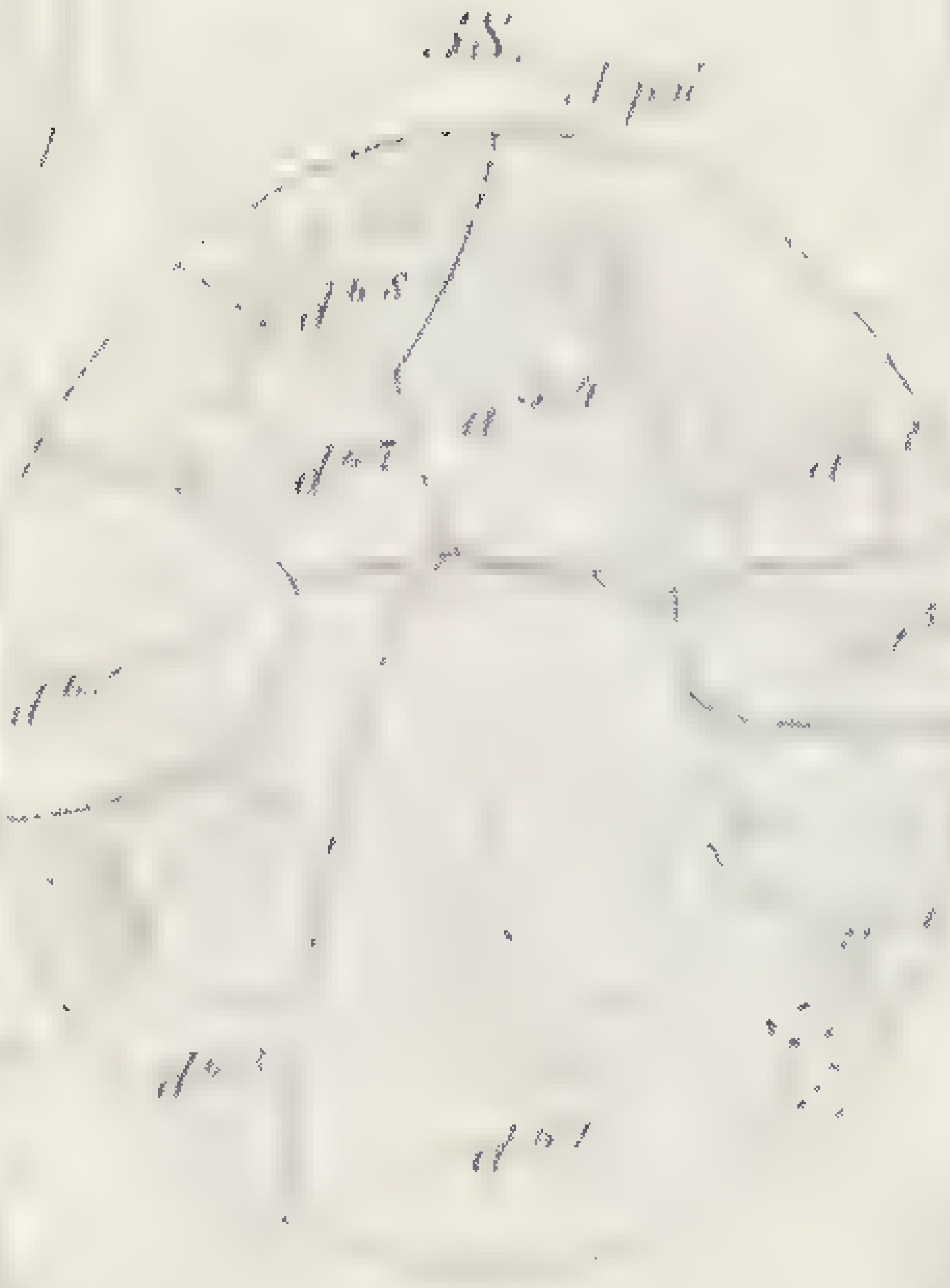
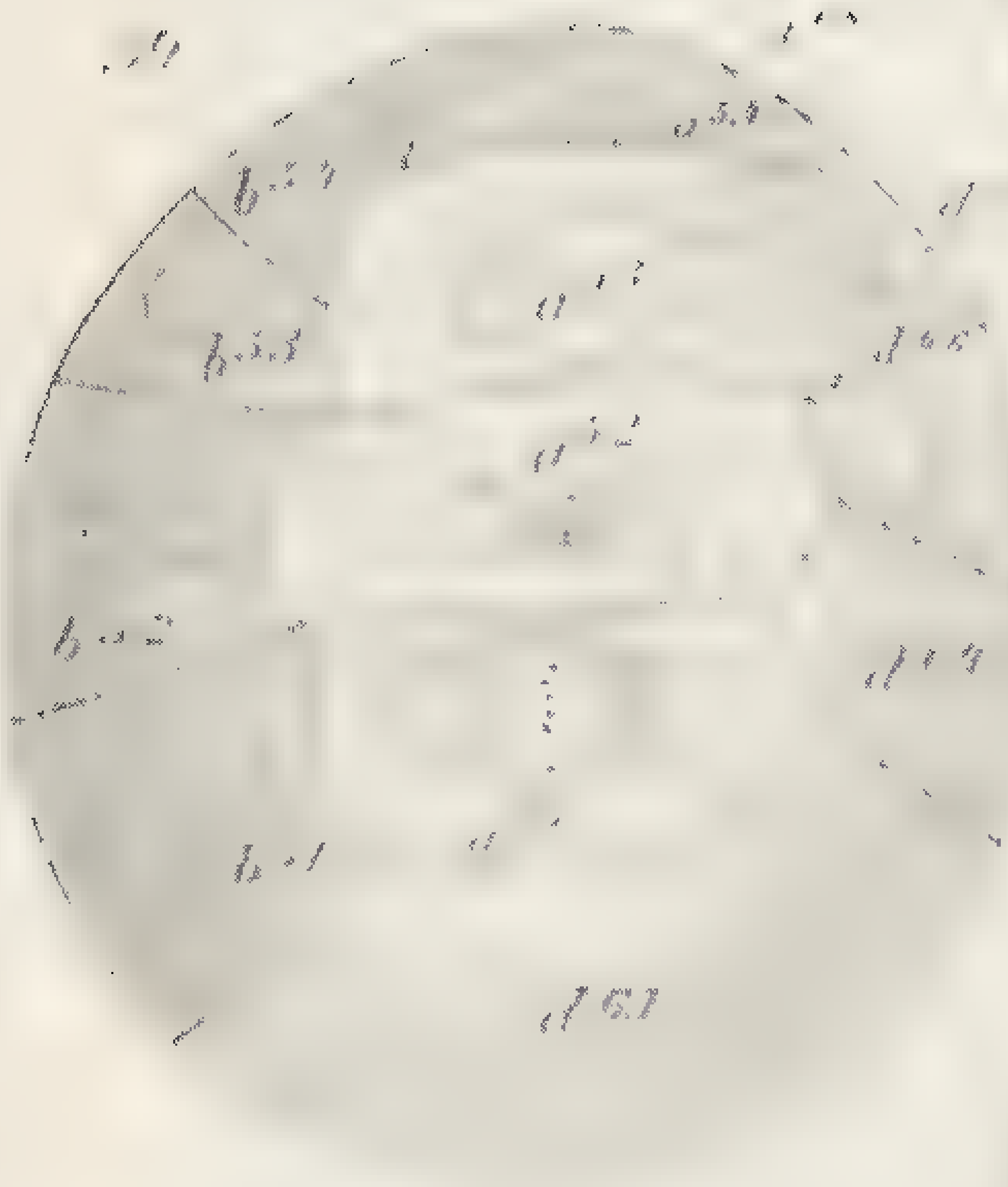
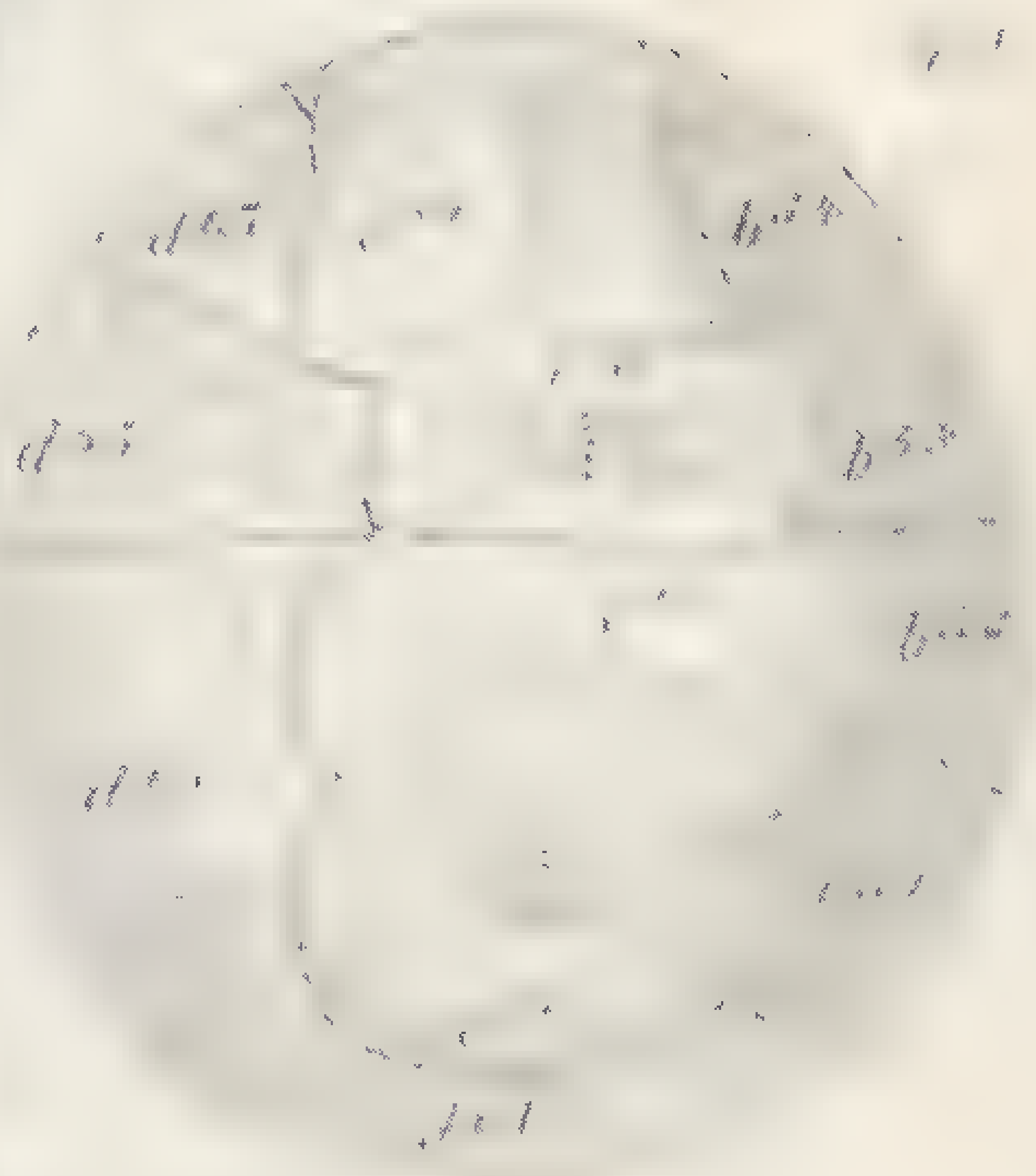
b 53
a 53
d 53
53


57
d 68
d 67
a 54
d 66
d 65
a 51
a 52
a 51
d 64
d 63
d 62


58
Apü
d 68
d 67
a 54
d 62
d 63
d 61


59
b 54
b 53
a 53
a 52
d 66
b 52
d 64
b 51
d 61


60
d 67
b 54
d 5
b 53
b 52
d 61

61

PLATE 6.

Fig. 44. Right side of the egg shown in Figure 43, Plate 5, 27-cell stage.

Fig. 45. Dorsal pole of the egg shown in Figures 43 and 44.

Fig. 46. Posterior view of the egg shown in the three preceding figures. Note the lateral position of the asters in $d^{6\cdot3}$.

Fig. 47. Right anterior surface of an egg, showing the quadrants B and C in the sixth generation.

Fig. 48. Sagittal optical section of the 32-cell stage, viewed from the right side, and showing the spindles in $d^{5\cdot1}$ and $d^{6\cdot3}$.

Fig. 49. Sagittal optical section of an egg slightly older than that seen in Figure 49, showing the process by which the cell $d^{7\cdot2}$ is formed at the sixth cleavage of the entoderm.

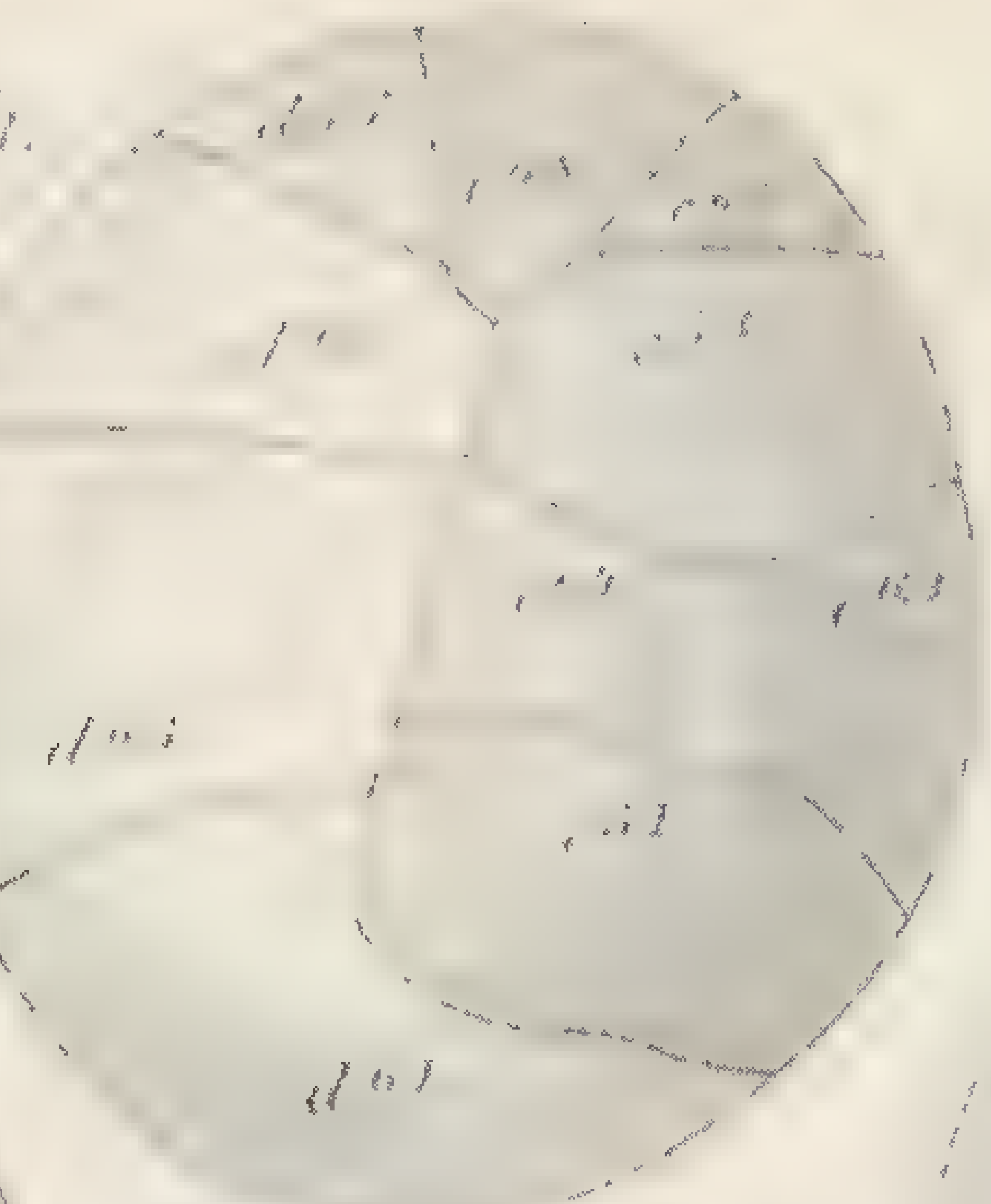
Fig. 50. Sagittal optical section of an egg a little older than that seen in Figure 49, showing the recently divided and separating asters in $d^{7\cdot1}$, and the beginning of migration of the cloud of granules which lies at the anterior ventral margin of the cell $d^{7\cdot1}$.

Fig. 51. Sagittal optical section of about the same stage as that shown in Figure 50. The nucleus of $d^{7\cdot1}$ has moved away from the periphery of the cell, and the cloud of granules is distributed between it and the small cells $d^{5\cdot2}$ and $d^{7\cdot2}$.

Apparently there is some slight variation in regard to the changes in the entoderm cell as compared with the other cells. From the condition of the remaining cells of the D quadrant, one would infer that Figure 51 is younger than Figure 50, though the migration of the cloud of granules is more advanced.

Fig. 52. Transverse optical section of the egg shown in Figure 51, through the region marked in Figure 51 by the cell $d^{5\cdot2}$. The section is viewed from the ventral side.

44.



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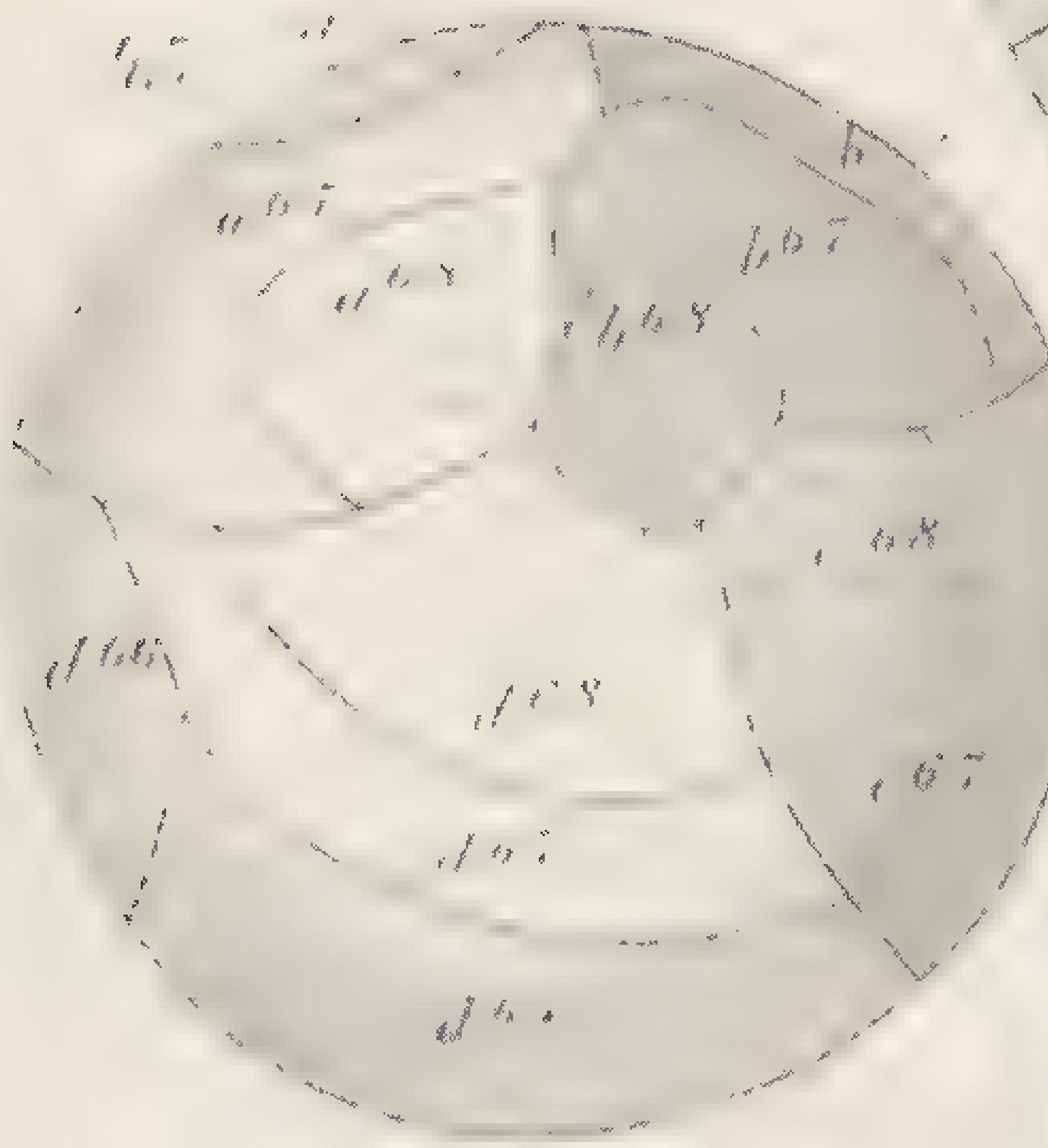
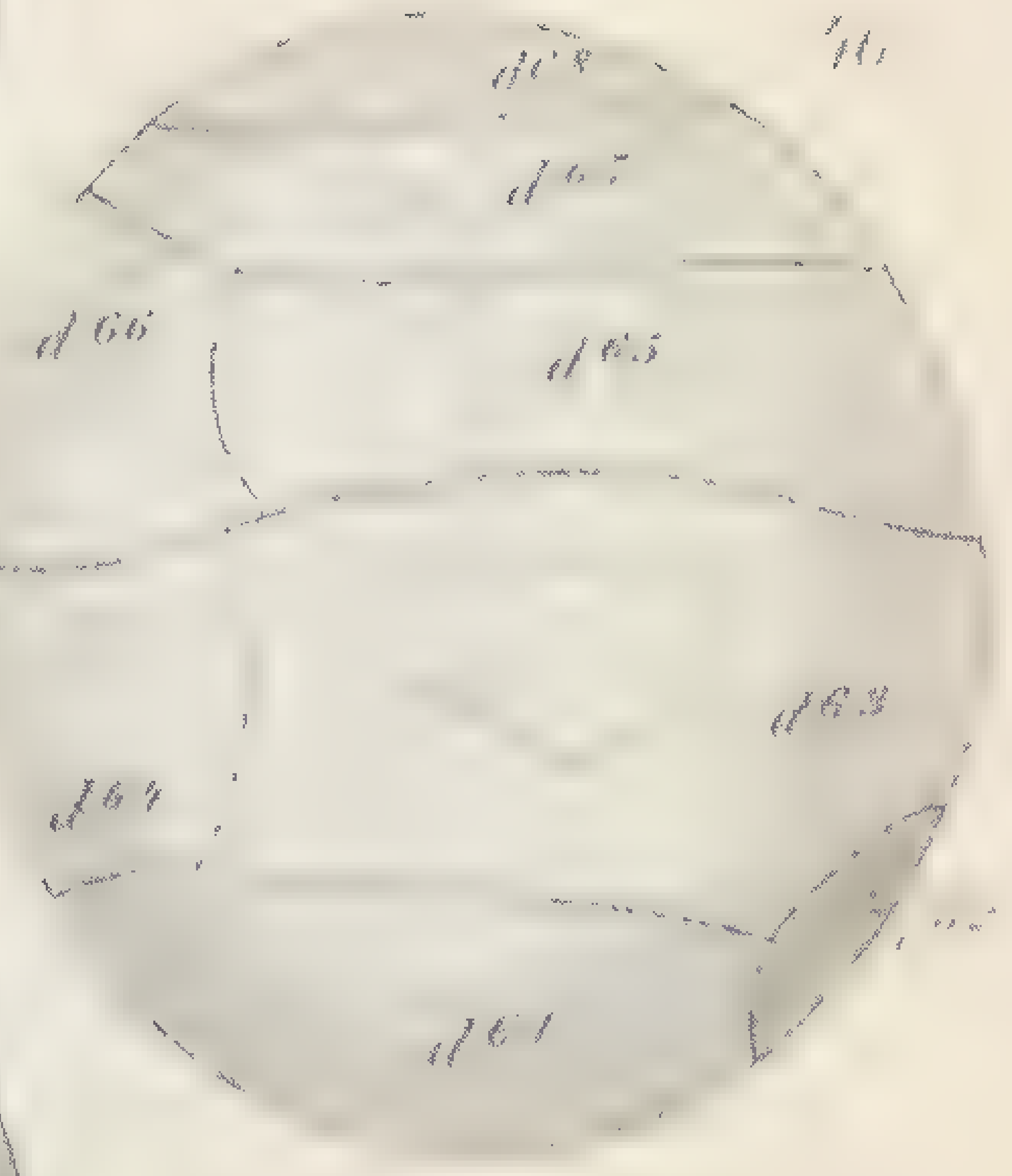


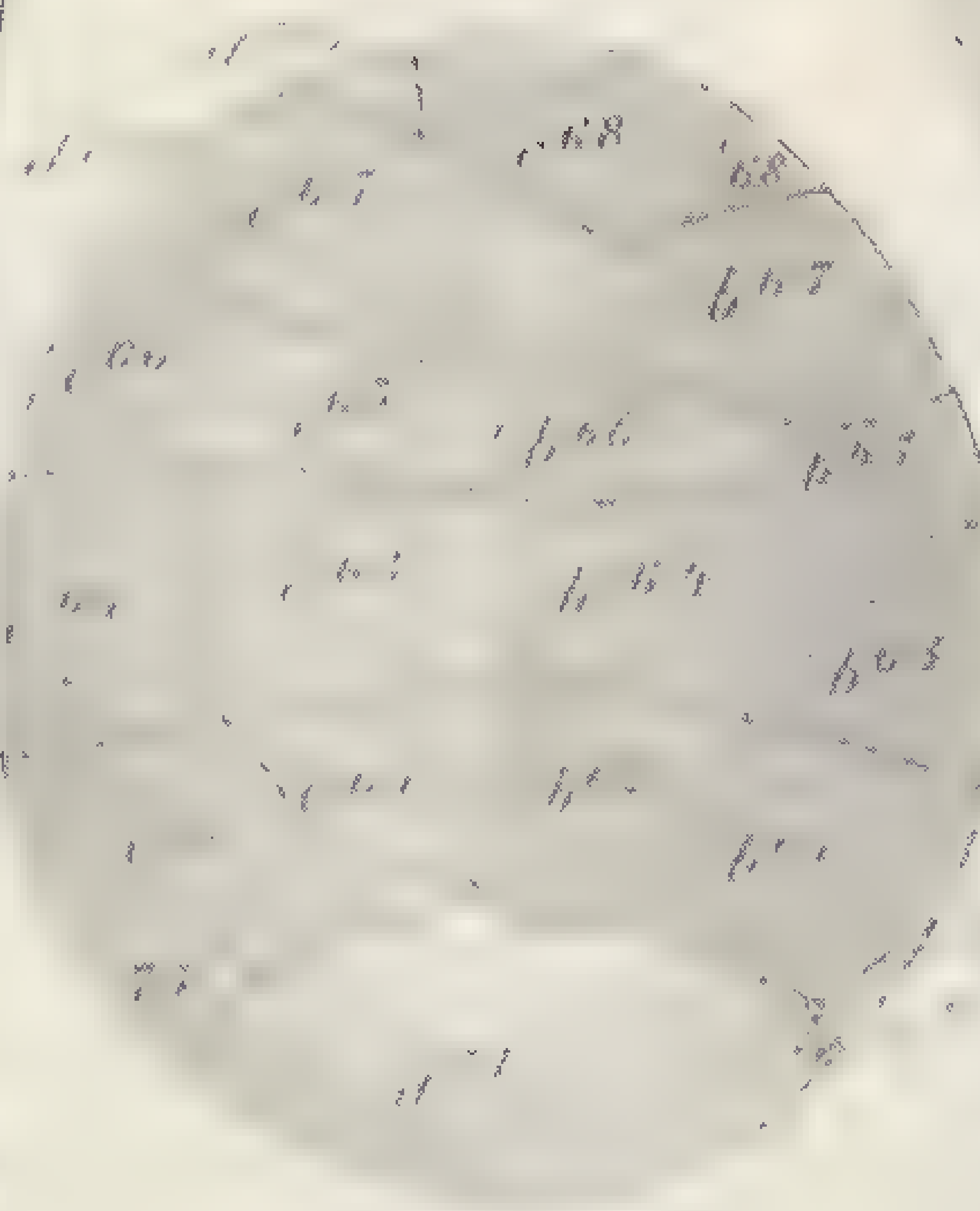
b67
b68
c68
d66
d68
c67
d64

46.



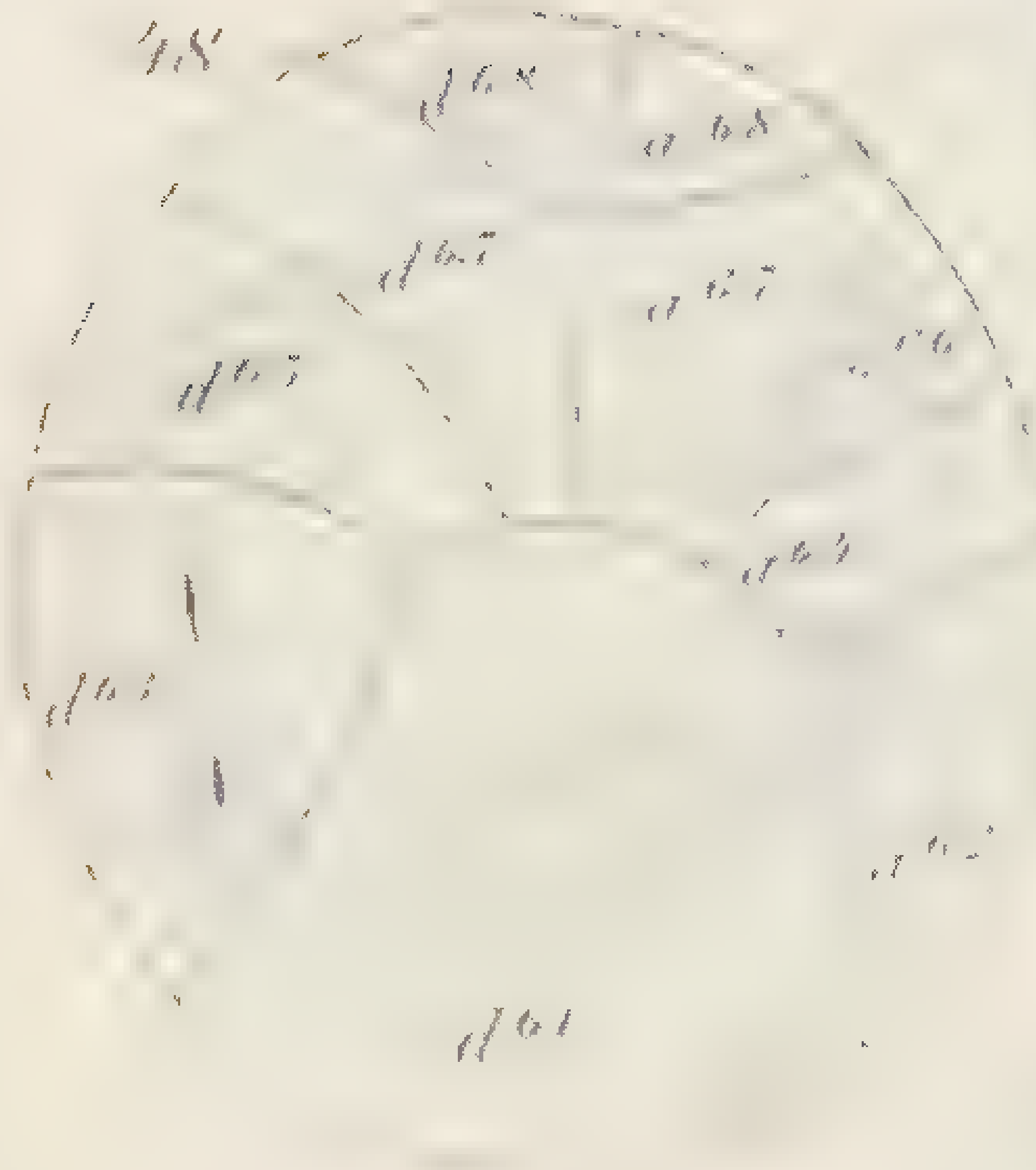
d68
d67
d66
d65
d63
d64
d61

47.



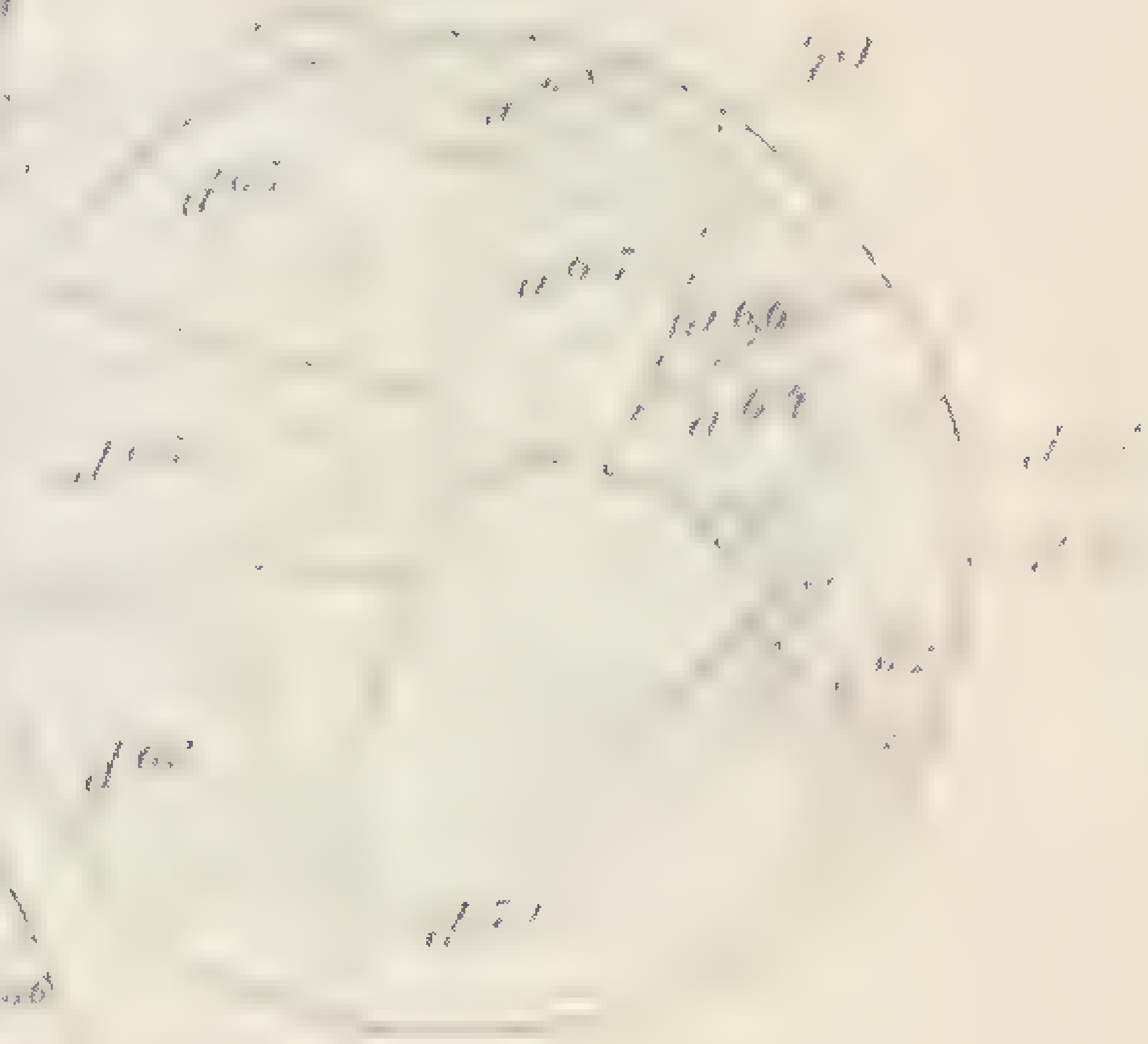
dpd
c68
b67
b66
b64
b63
d71

48.



d68
a68
d67
a67
d65
d61

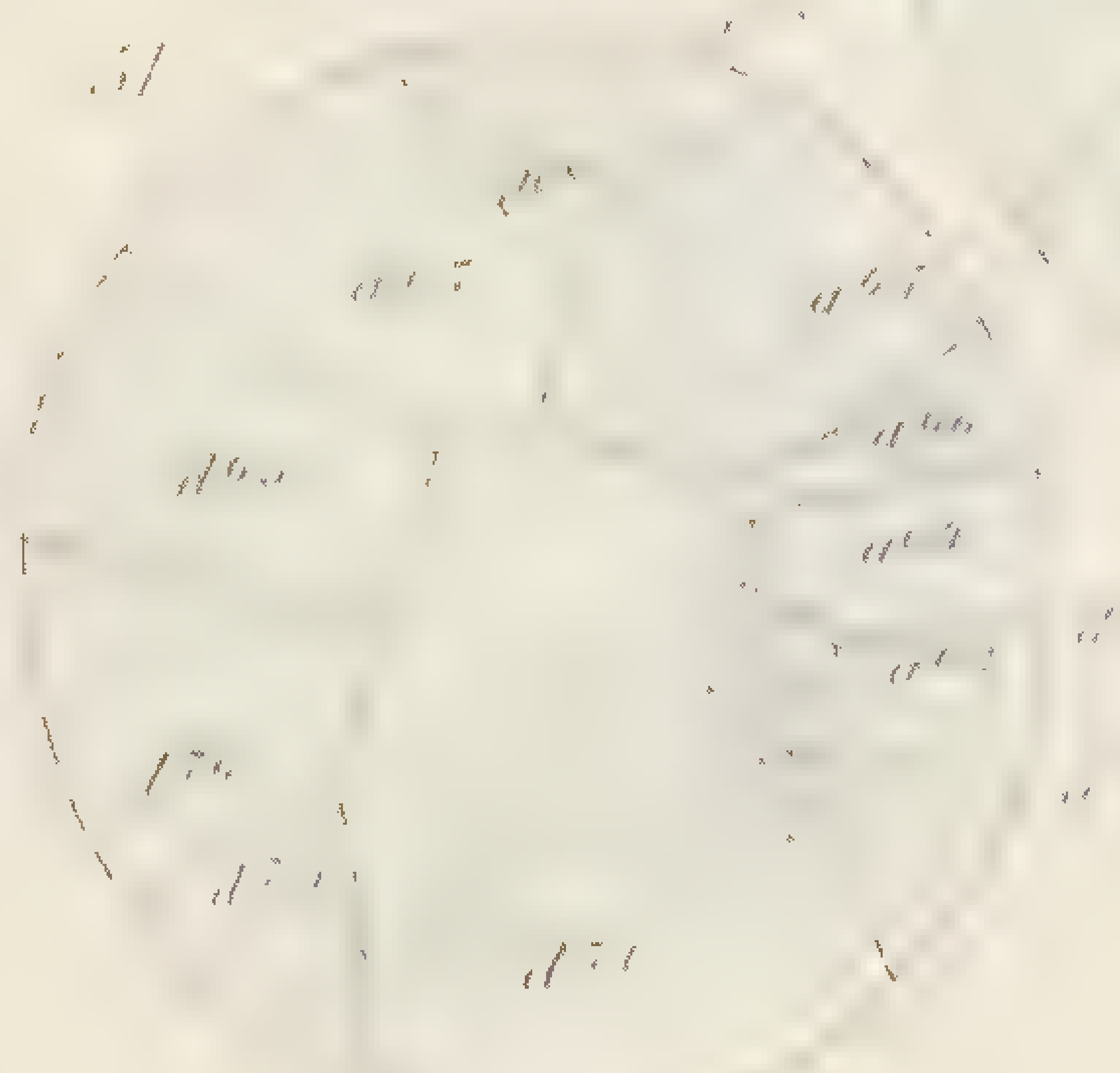
49.



a67
b66
a64
d71

50.

51.



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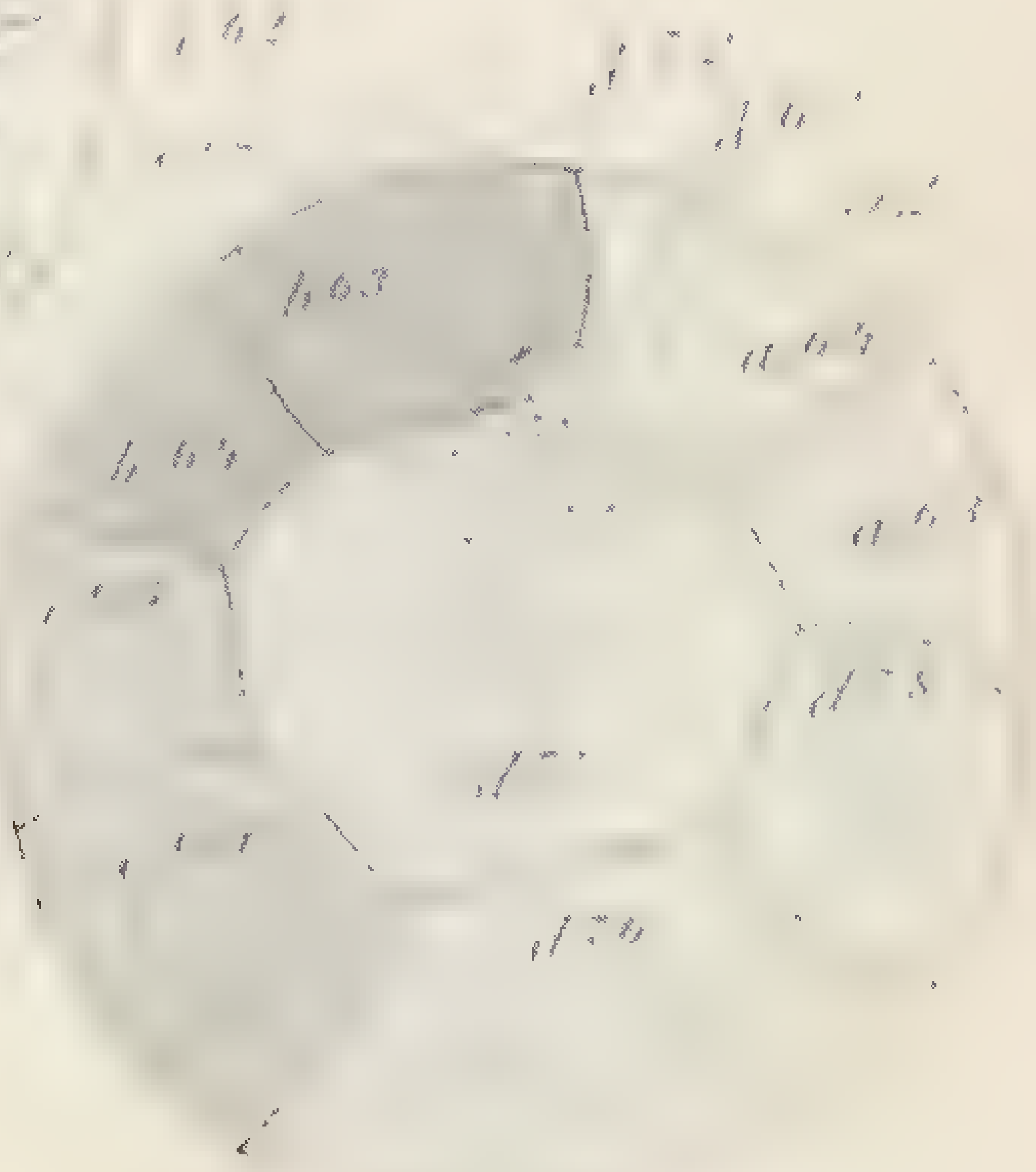


b63
b64
a64
a63
d73
d76

PLATE 7.

Fig. 53. Quadrant *D* at a stage a little older than that shown in Plate **6**, Figure 46. The rotation of the asters in the cell $d^{6 \cdot 8}$ is in progress. Thirty-two cells.

Fig. 54. Later stage than Figure 53, showing the final dorso-ventral direction of the spindle in $d^{6 \cdot 3}$. A section of this egg is represented in Plate **6**, Figure 48. Thirty-two cells.

Fig. 55. Anterior surface of 49-cell stage, showing the sixth cleavage in progress.

Fig. 56. Ventral end of the egg seen in Figure 55, showing the large cell $d^{7 \cdot 1}$ nearly enclosed by the other cells.

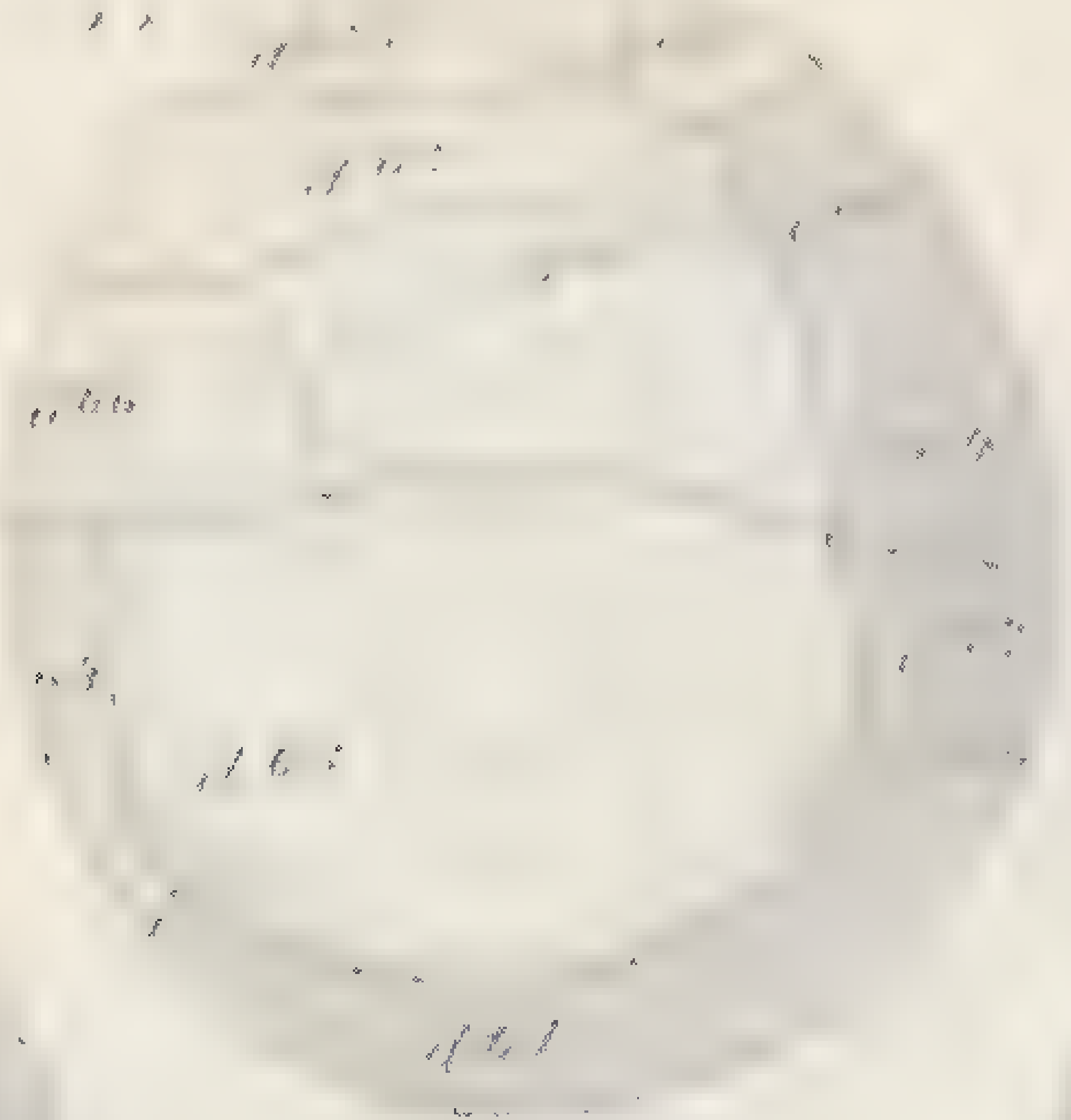
Fig. 57. The sixth cleavage in quadrant *D*. 38-cell stage.

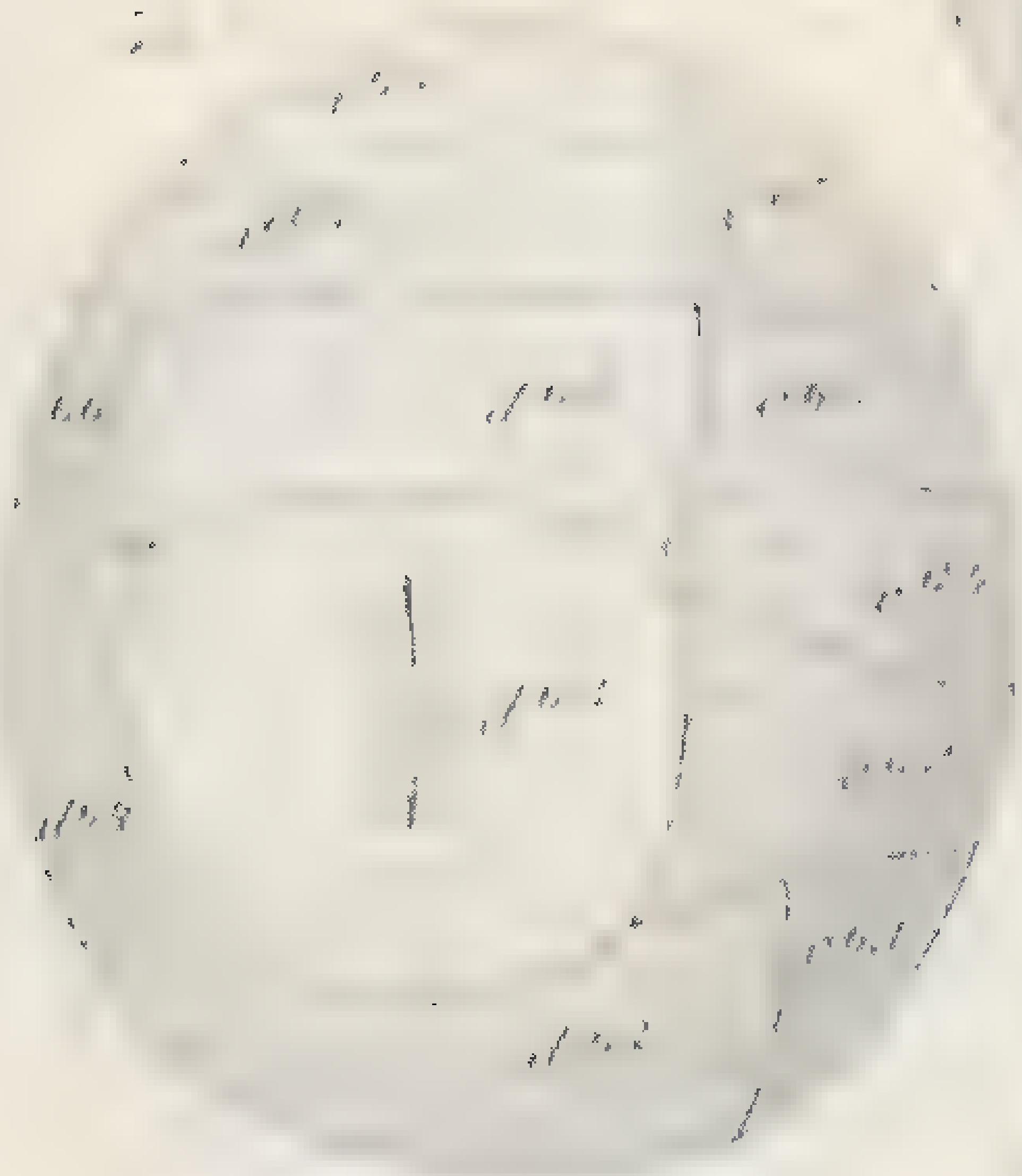
Fig. 58. Completion of the sixth and beginning of the seventh cleavage in quadrant *D*. Same egg as that shown in Figures 55 and 56.

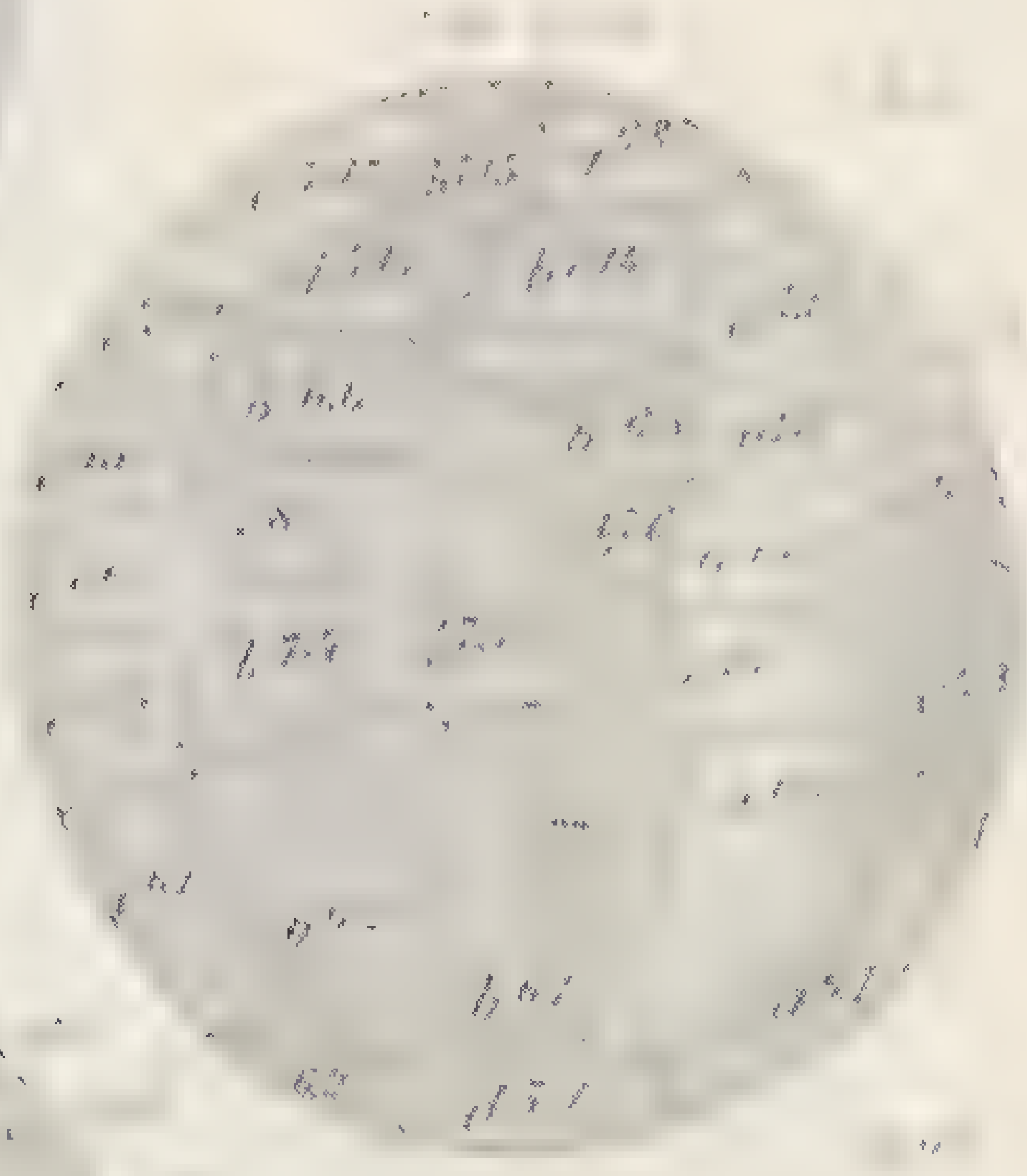
Fig. 59. Dorsal view of the egg seen in Figure 57.

Fig. 60. Dorsal view of a stage later than the preceding, representing the same egg as that shown in Figure 58. Sixth cleavage nearly completed.

Fig. 61. Anterior view, showing the completion of the sixth cleavage in the quadrants *A* and *B* in all the cells except $a^{6 \cdot 5}$ Sixty-nine cells.







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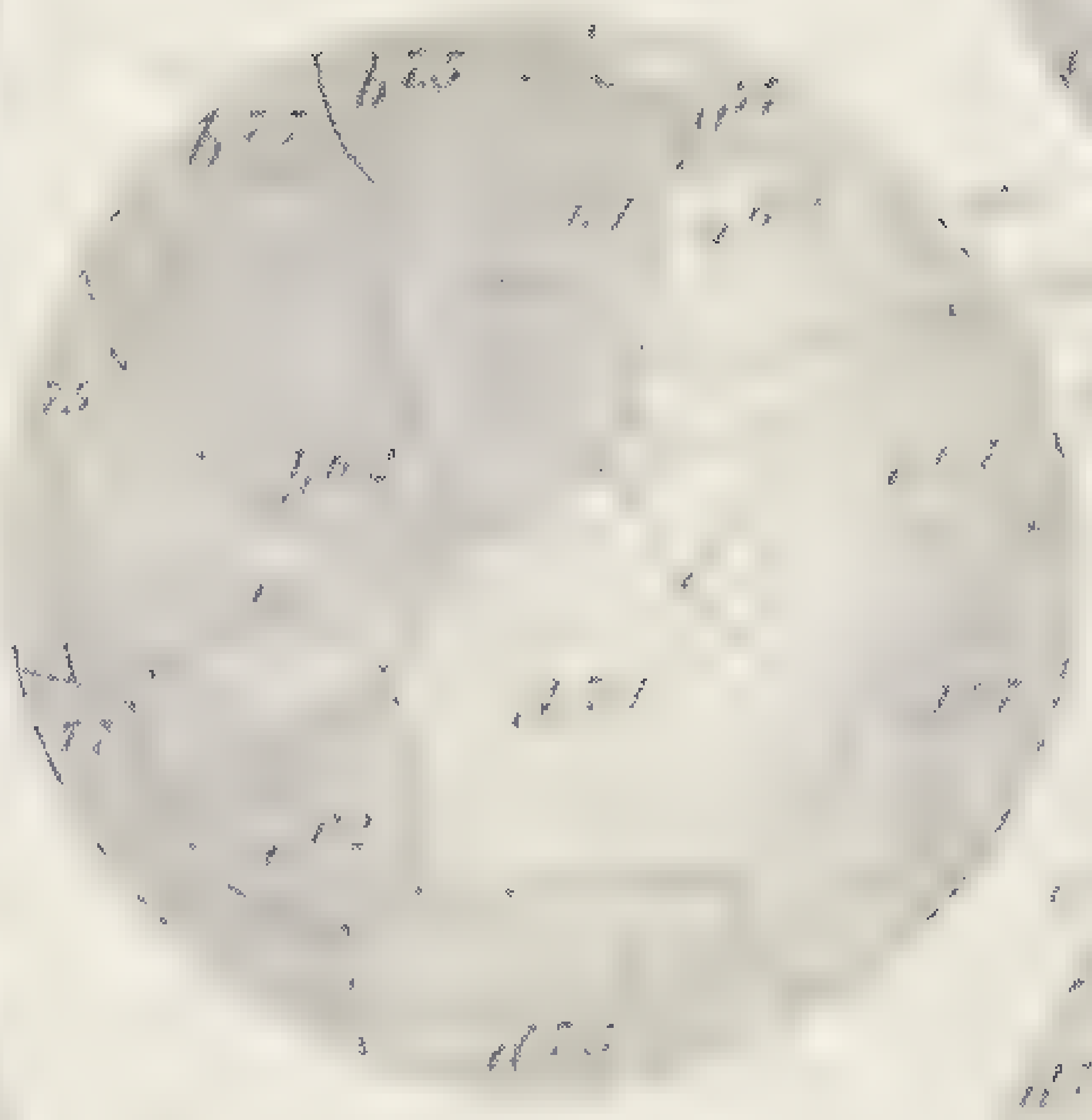

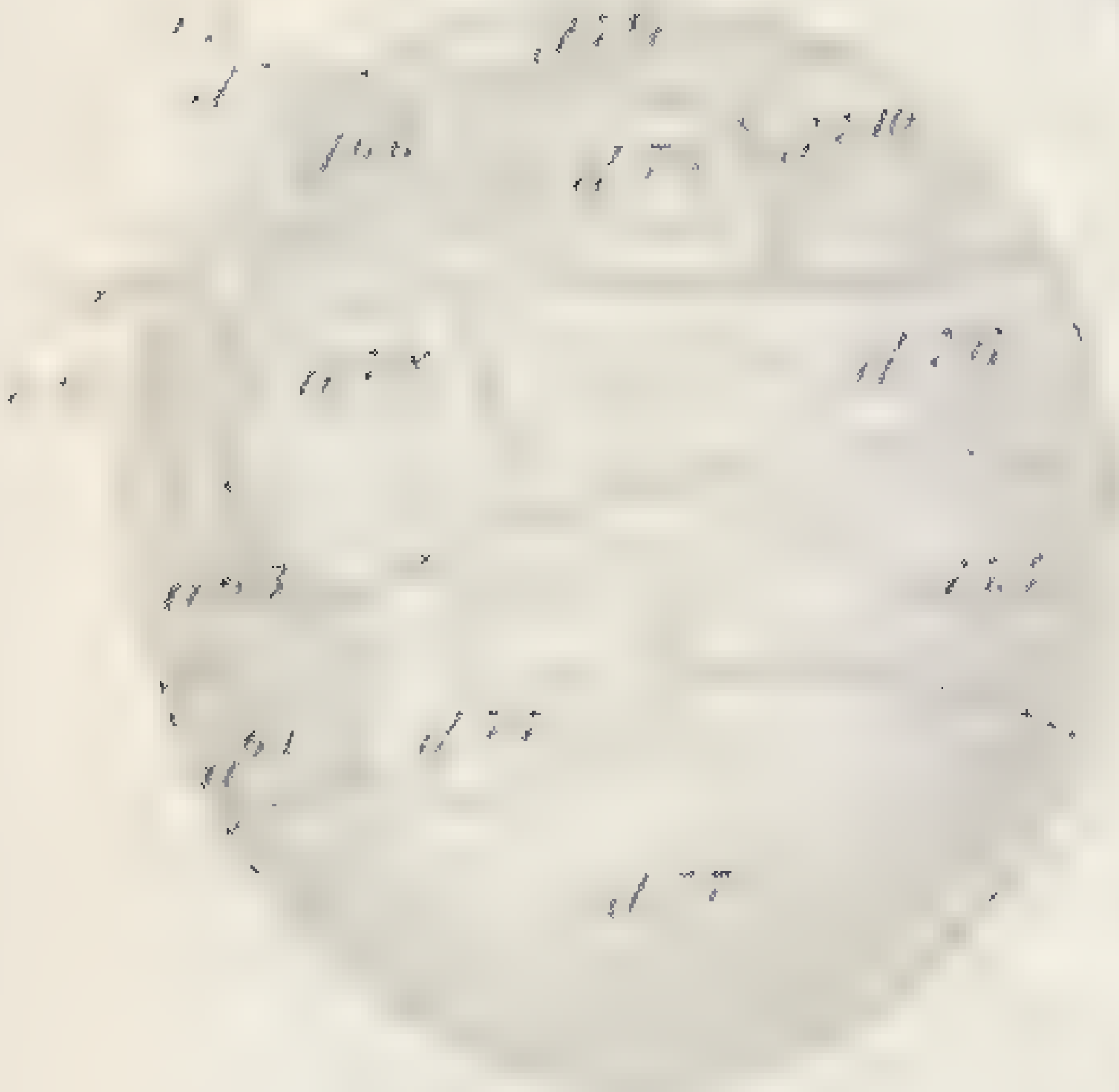

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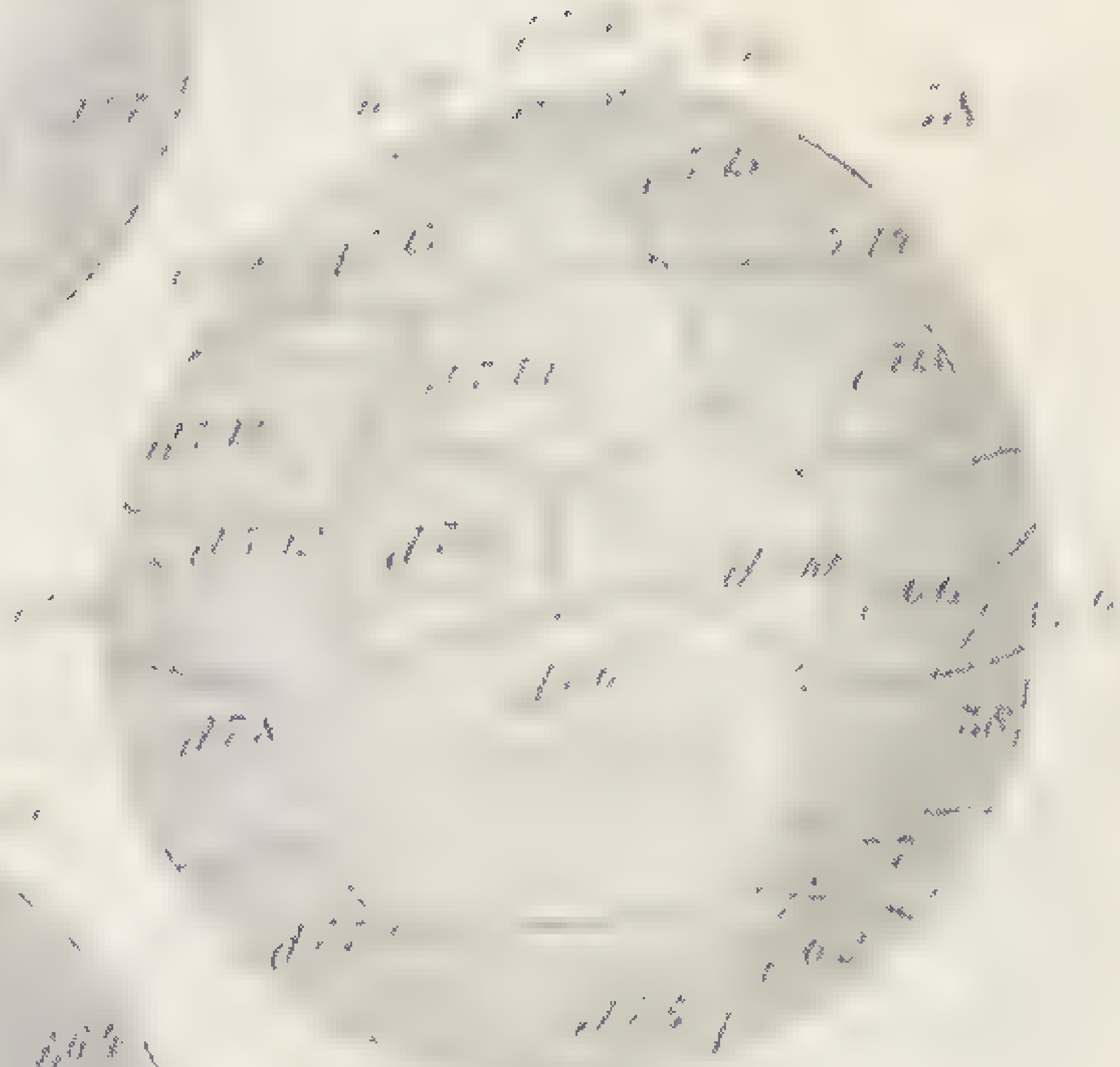

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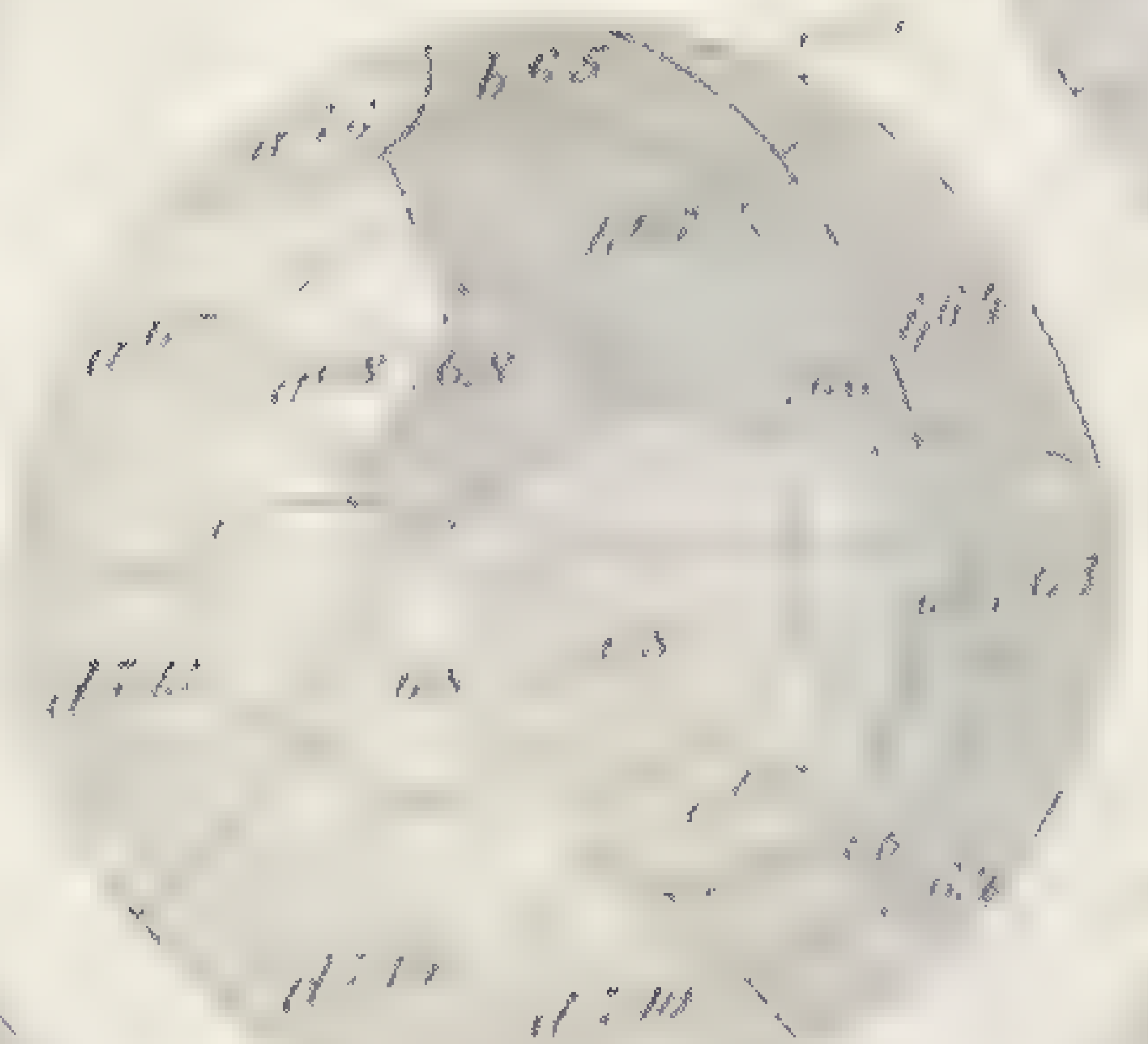

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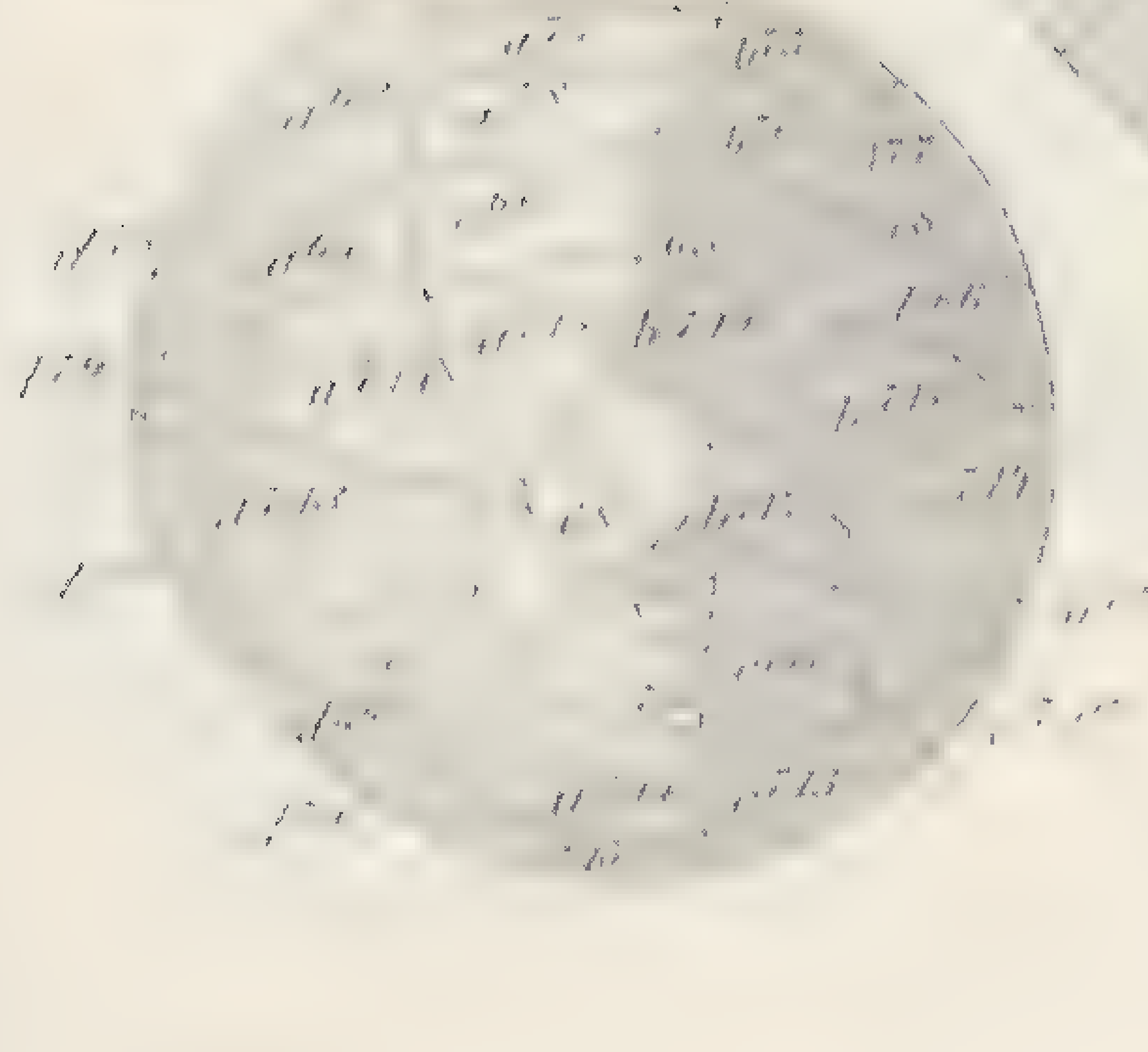

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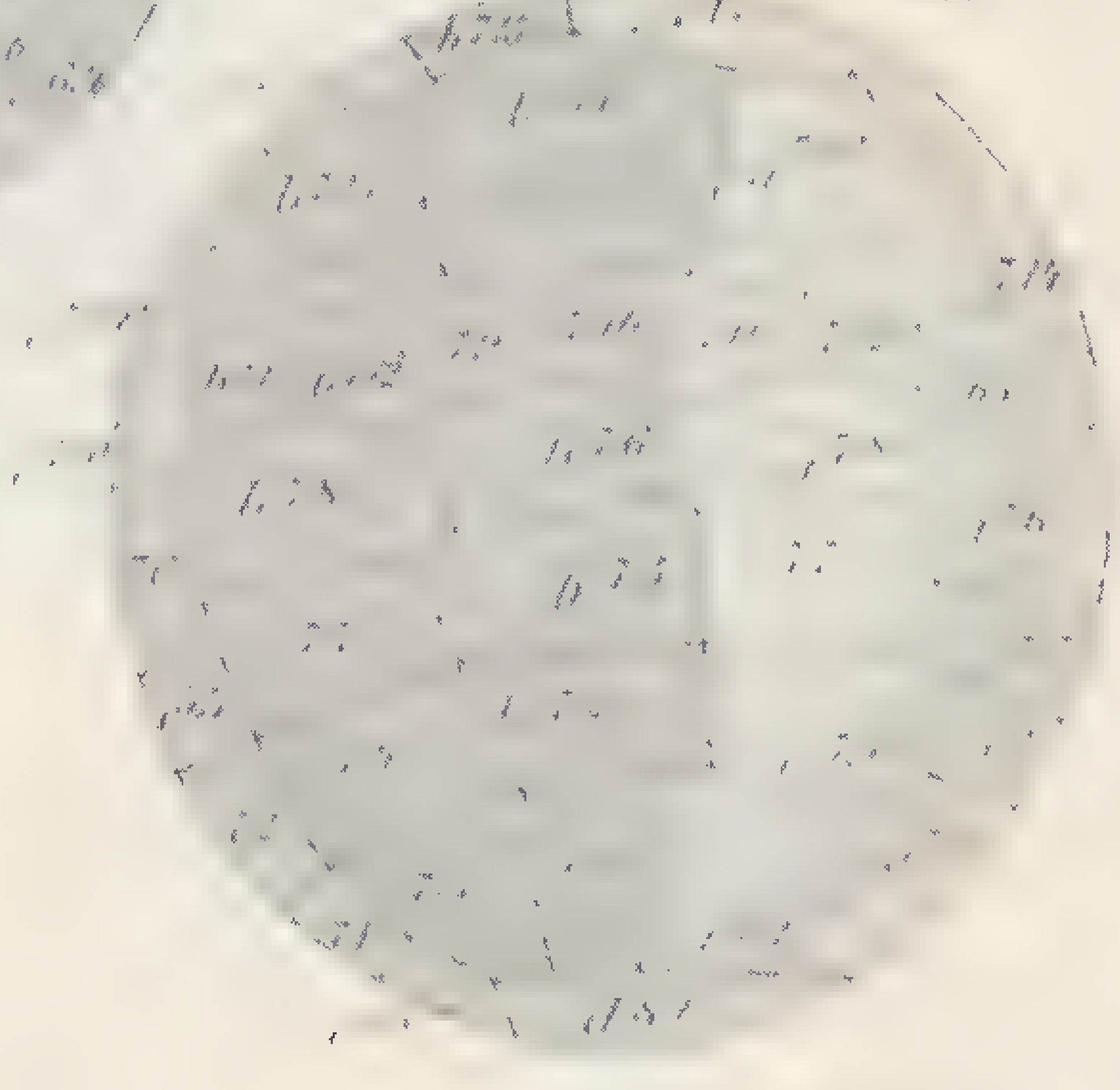


PLATE **8**.

Fig. 62. Dorsal view of an egg in approximately the same stage as that of Plate **7**, Figure 61. The four small unlabelled cells at the point of meeting of the four quadrants are $a^{7\cdot 16}-d^{7\cdot 16}$. About 69-cell stage.

Fig. 63. Ventral view of the egg shown in Figure 61, exhibiting the nearly complete enclosure of the entoderm (to which the cell $d^{8\cdot 1}$ belongs) by the ectoderm.

Fig. 64. Sagittal optical section of the egg represented in Plate **7**, Figures 55, 56, and 59. The cloud of granules in the entoderm cell $d^{7\cdot 1}$ now surrounds the dorsal aster of the spindle of the seventh cleavage.

Fig. 65. Sagittal optical section of the egg represented in Figures 61 and 63, showing the completed seventh cleavage in the entoderm; the cloud of granules forms a ring around the nucleus in the smaller entoderm cell $d^{8\cdot 2}$. The ring being cut twice, appears in the form of two groups of granules, one anterior, the other posterior to the nucleus.

Fig. 66. Posterior view, showing the seventh cleavage in quadrant D.

Fig. 67. Left posterior view; the seventh cleavage nearly finished in quadrant D; the sixth not yet finished in quadrant A. From the egg represented in Figures 61, 63, and 65. Sixty-nine cells.

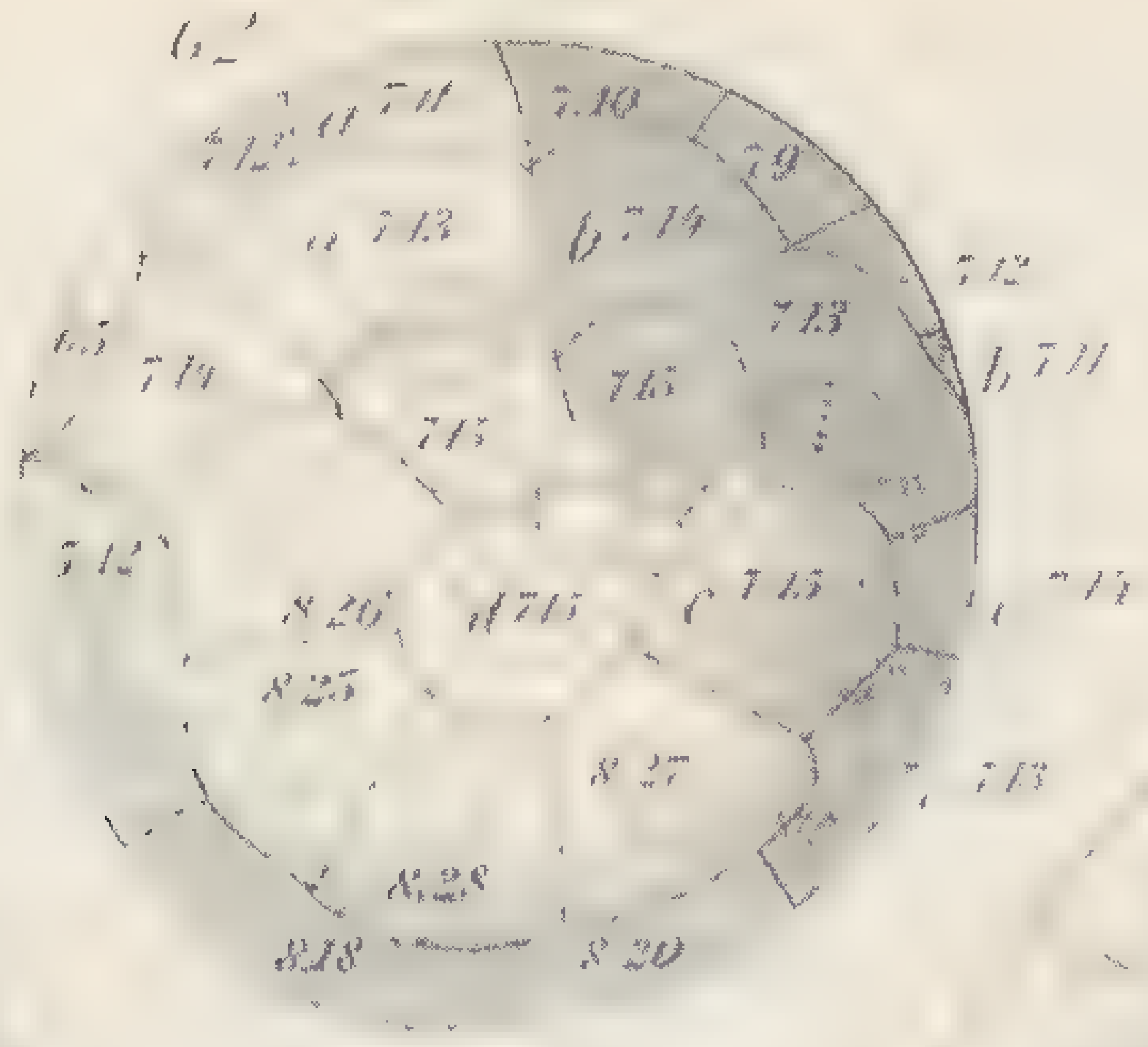
Fig. 68. Posterior view of a later stage than that represented in Figure 67. The line separating $d^{8\cdot 11}$ and $d^{8\cdot 12}$ is the posterior median line; the spindles are seen to be arranged symmetrically with respect to this line, so that the cleavage is becoming bilateral. The entoderm is entirely covered by the ectoderm; the region where $a^{7\cdot 1}$ and $d^{8\cdot 9}$ are in contact shows the place where the entoderm cells formerly occupied the surface. 82-cell stage.

Figs. 69–74 give different views of the same egg, a 94-cell stage.

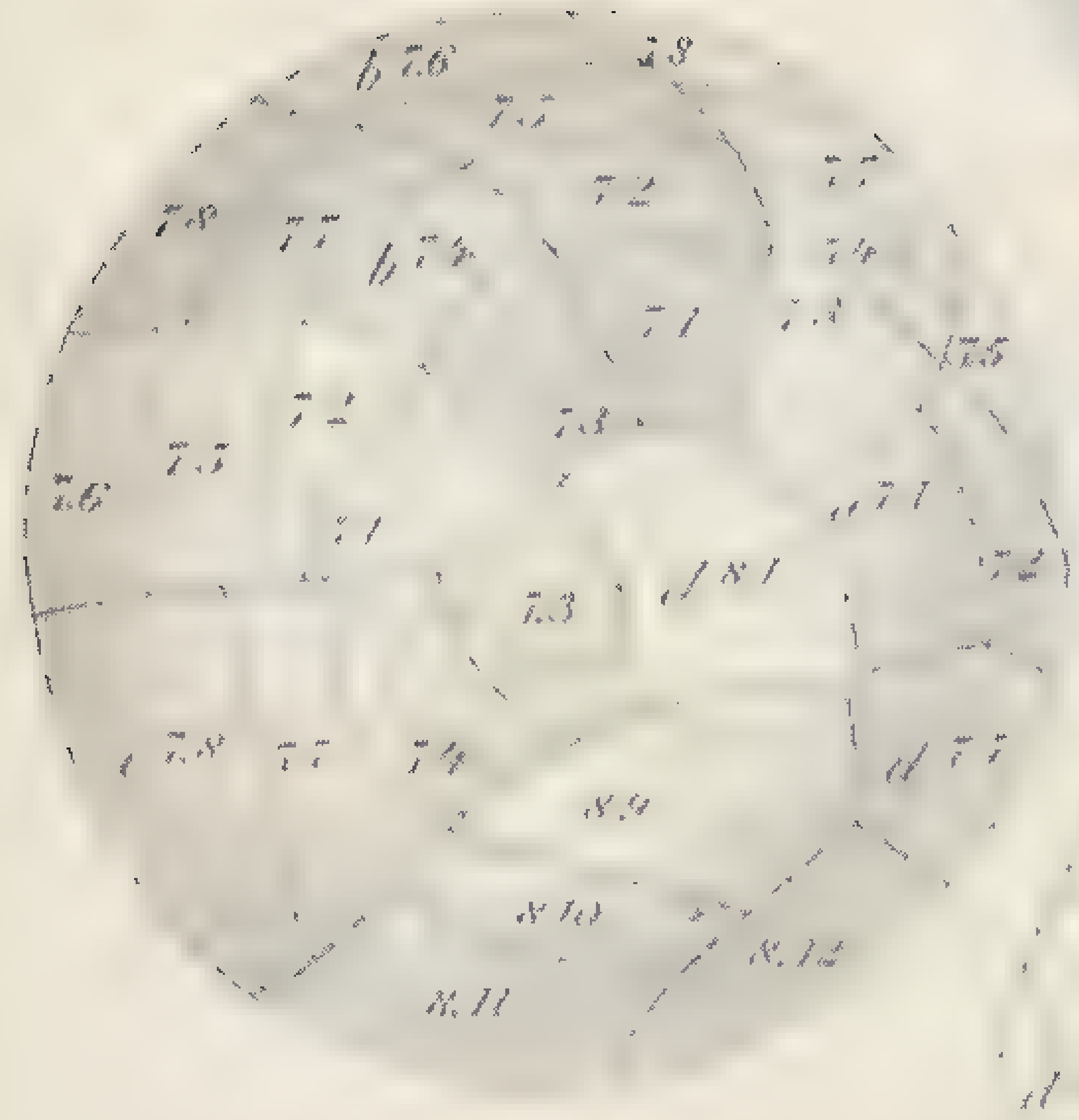
Fig. 69. Anterior surface, showing the finished meridional cleavage forming the cells $a^{8\cdot 11}-c^{8\cdot 11}$, $a^{8\cdot 12}-c^{8\cdot 12}$, $a^{8\cdot 15}-c^{8\cdot 15}$, and $a^{8\cdot 16}-c^{8\cdot 16}$, and the spindles for the equatorial cleavage of $a^{7\cdot 5}-c^{7\cdot 5}$ and $a^{7\cdot 7}-c^{7\cdot 7}$.

Fig. 70. Right side of the egg shown in Figure 69.

62


a 7 11
7.10
7.9
a 7.13
b 7.14
7.12
b 7.11
7.13
7.16
7.16
7.15
7.14
7.12
8.26
d 7.15
c 7.15
8.25
8.27
7.13
8.28
8.18
8.20

63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7.5
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b 7.4
7.4
7.1
7.3
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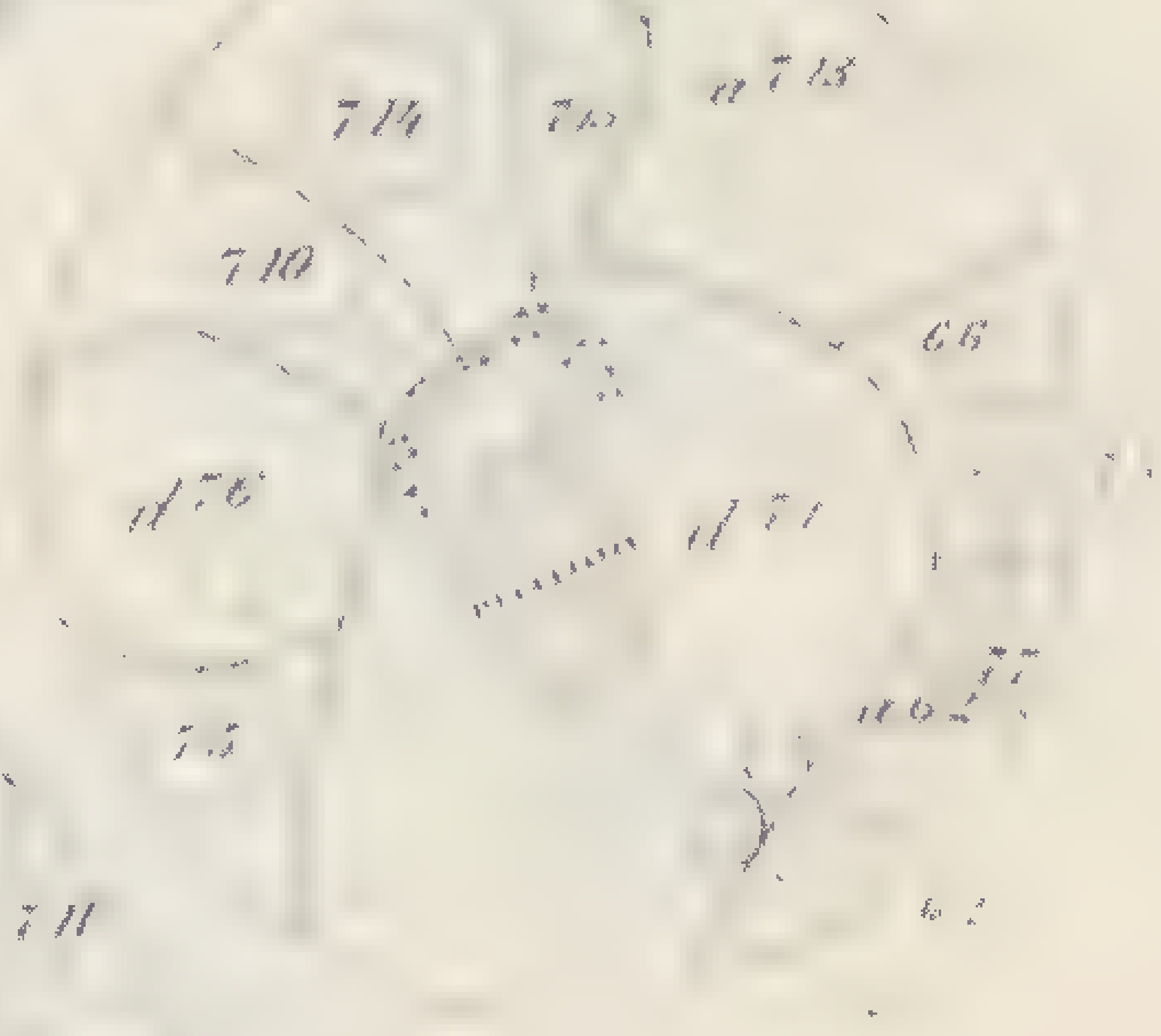
7.2
7.3
7.5
7.6
7.1
d 7.1
7.3
d 8.1
7.2
7.8
7.7
7.4
d 7.7
8.9
8.10
8.12
8.11

64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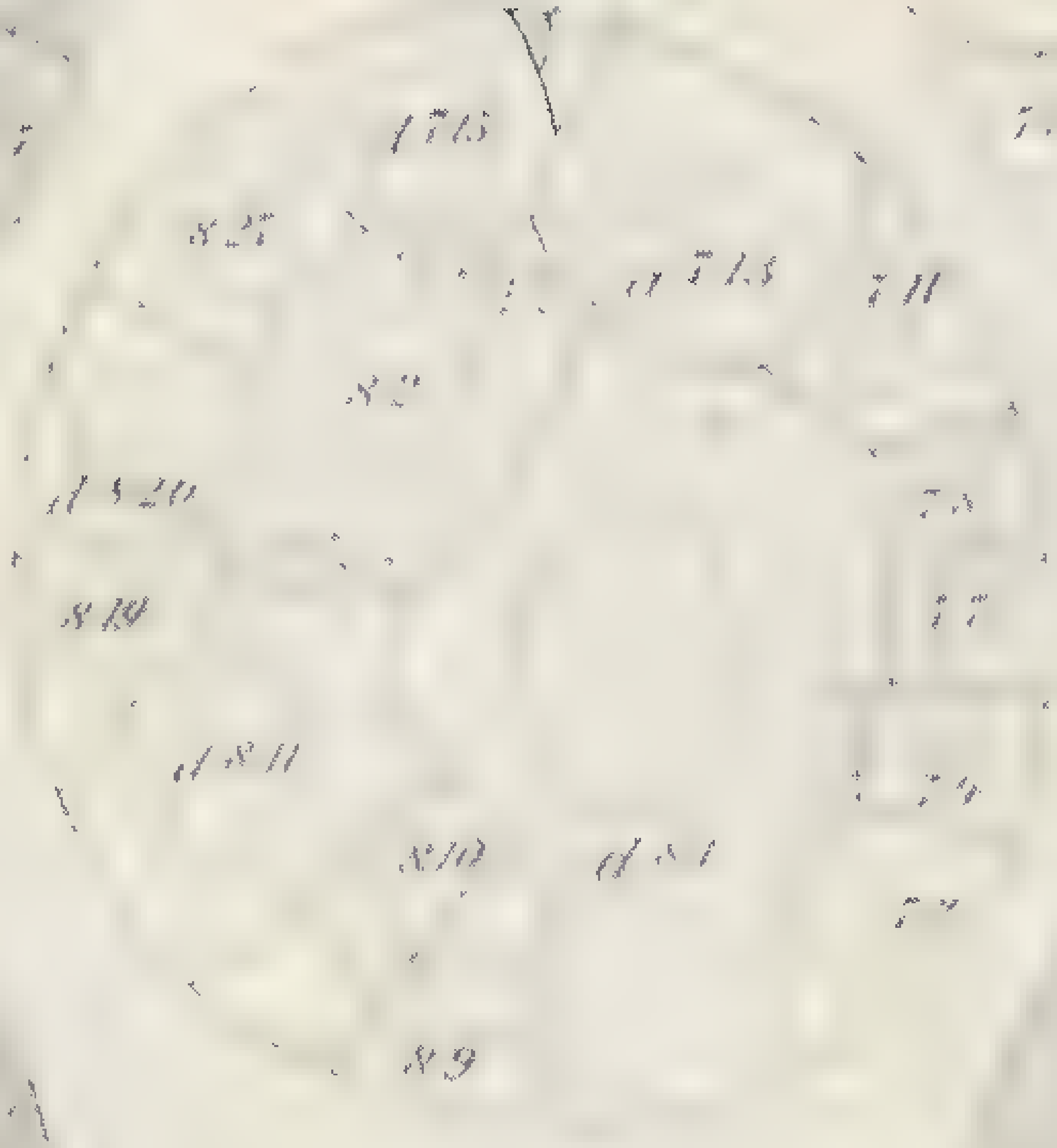
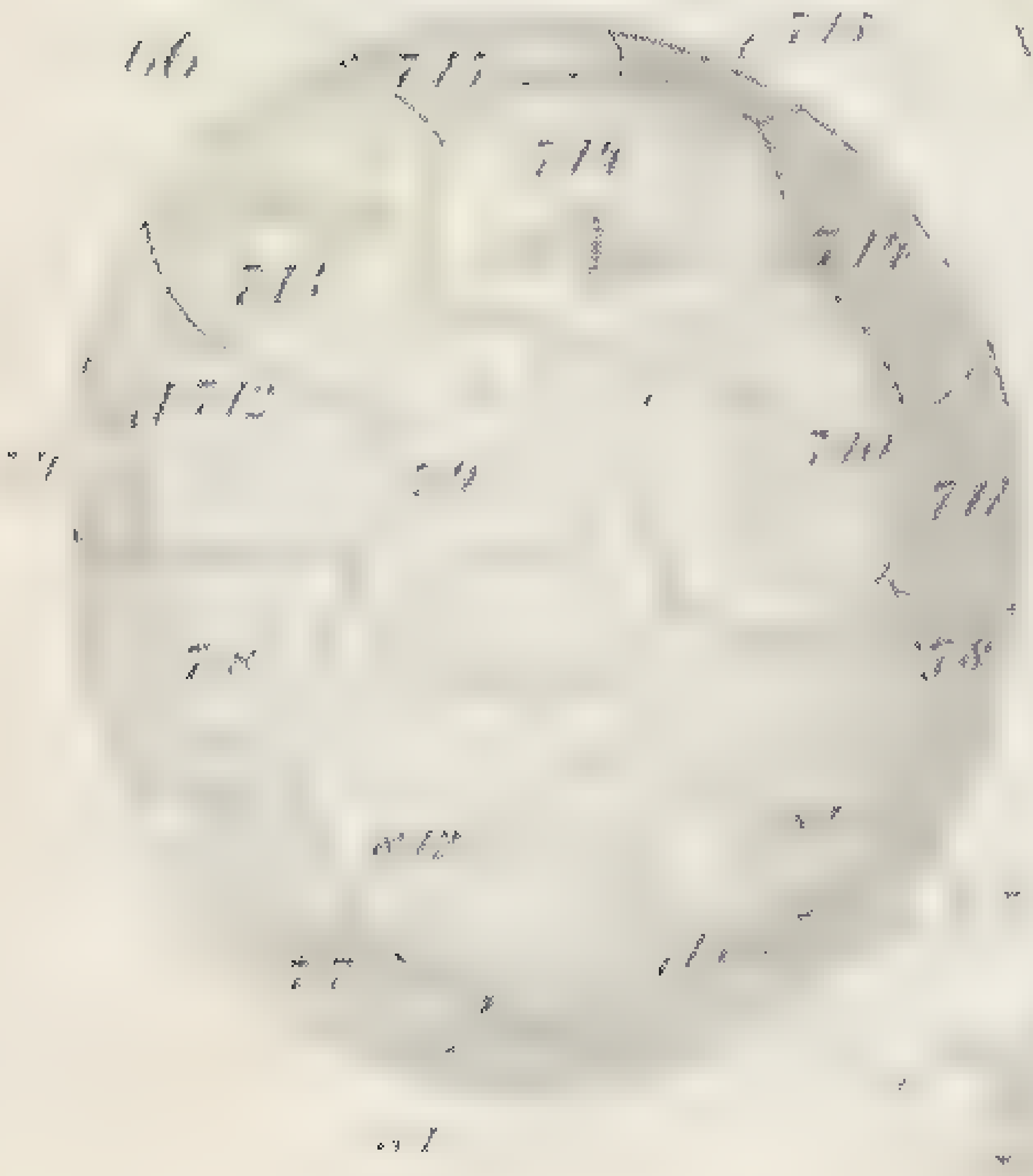
7.14
a 7.13
7.10
6.6
d 7.6
d 7.7
7.5
a 6.2

65


7.16
d 7.15
8.27
a 7.13
7.11
8.2
d 8.20
8.19
7.7
d 8.11
8.10
d 8.1
8.9

66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d 7.12
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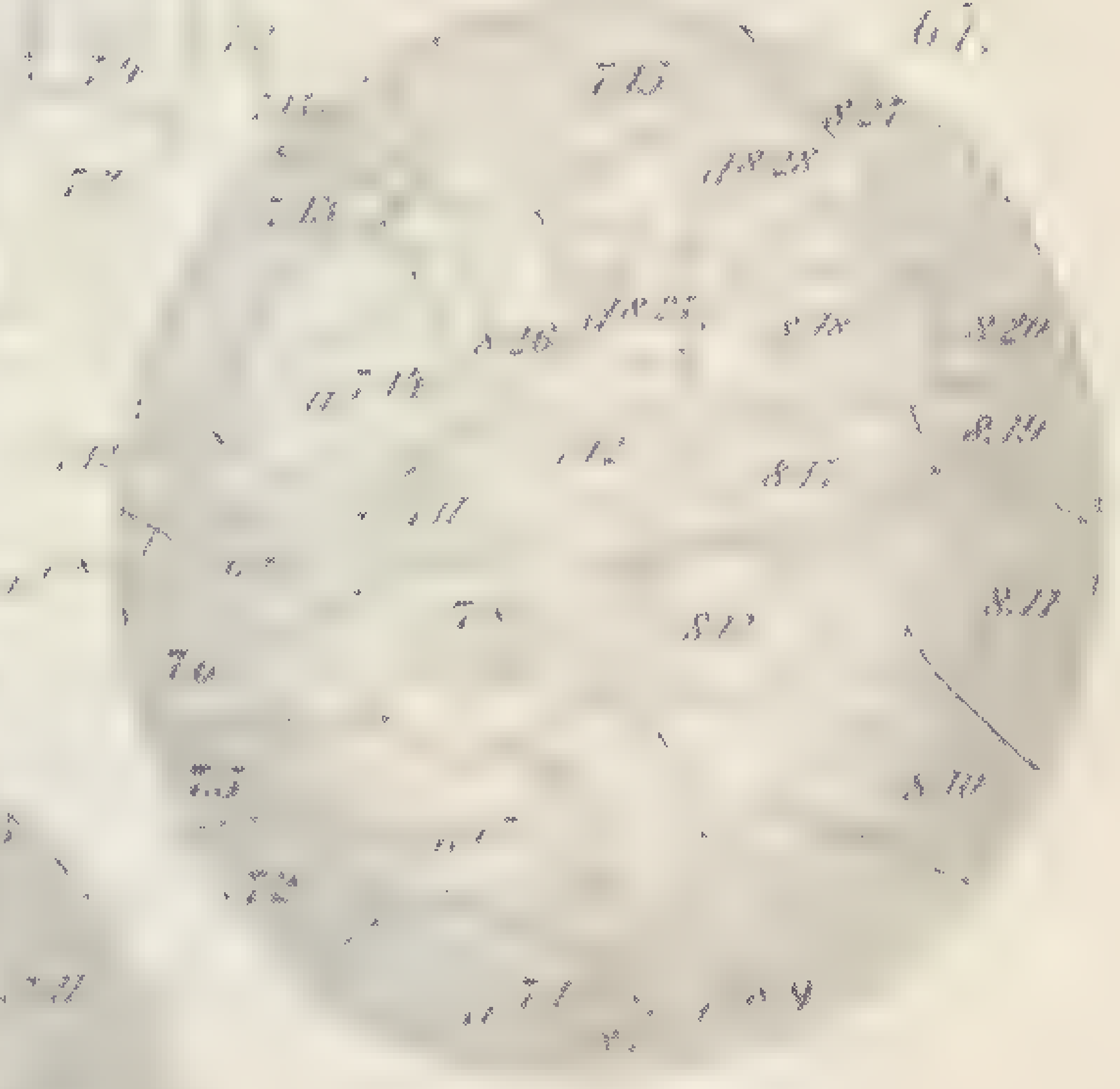
7.10
7.11
7.8
7.8
8.12
7.7

67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d 8.28
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8.26
d 8.25
8.18
8.20
a 7.14
8.19
8.17
8.11
7.6
8.10
7.5
7.2
a 7.1
8.9

68

8.27
d 8.28
8.25
8.26
8.18
8.17
7.2

69

70

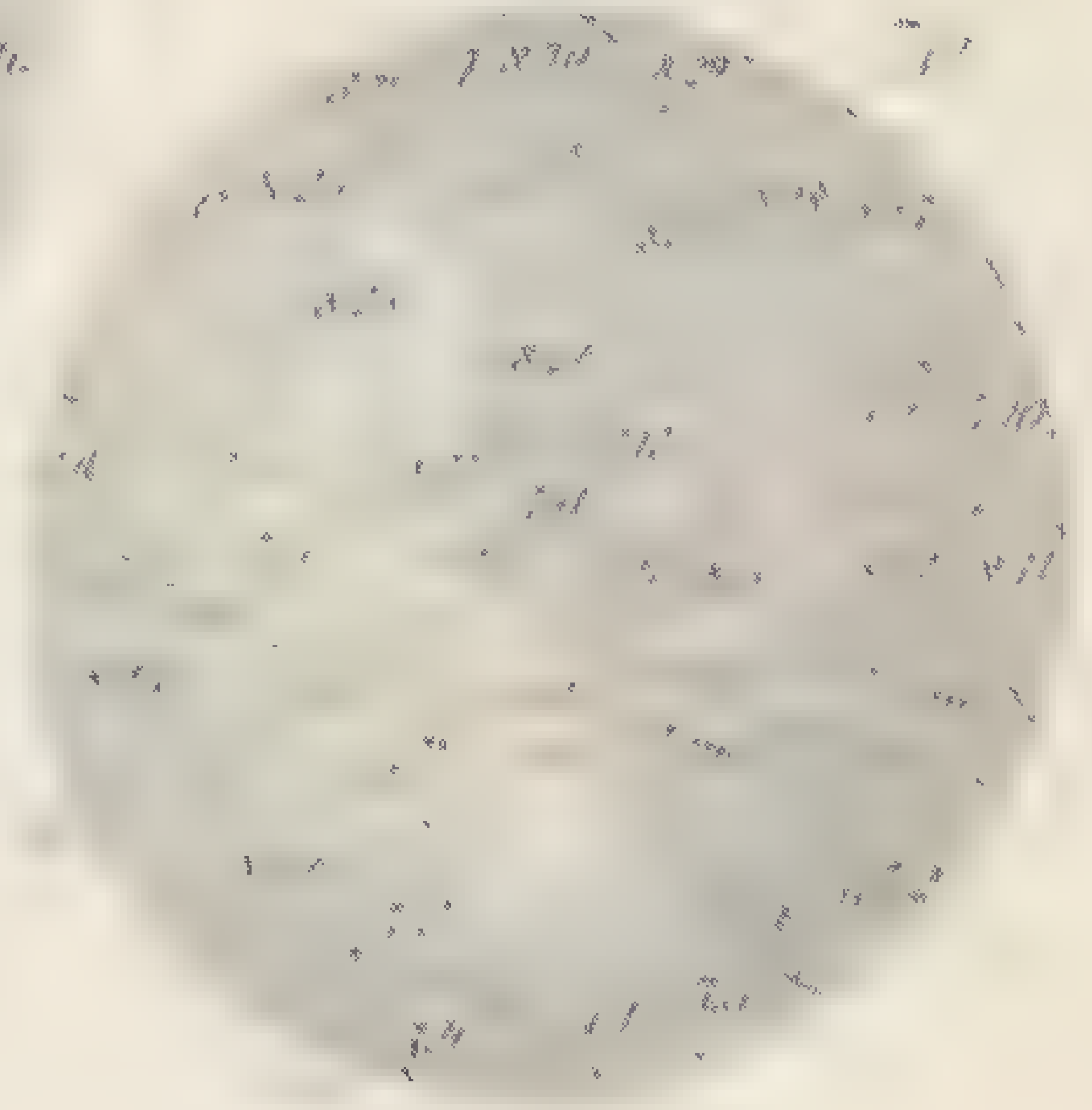

8.20
8.19
7.10
8.11

PLATE 9.

Fig. 71. Left side of the egg shown in Figures 69–74. Ninety-four cells.

Fig. 72. Dorsal view of the egg represented in Figures 69–74. The small cells in the centre, at the point of meeting of the four quadrants, are $a^{7\cdot16}-d^{7\cdot16}$.

Fig. 73. Ventral end of the same egg, showing the crowding together of the cells of the quadrants *A*, *B*, and *C* in this region.

Fig. 74. Posterior view of the same egg, showing spindles for the ninth cleavage in some of the cells of quadrant *D*, and the ninth cleavage completed in other cells of that quadrant.

Fig. 75. Anterior surface of a later stage, containing about 120 cells. At the ventral end (lower part of the figure) the cells are much crowded and many of them are very small. The vesicles immediately below the cells $b^{8\cdot15}$, $a^{8\cdot10}$, and $a^{8\cdot14}$ are the small ventral products of the cleavage of $a^{7\cdot5}-c^{7\cdot5}$ and $a^{7\cdot7}-c^{7\cdot7}$, the spindles for which are shown in Figures 69 and 70, Plate **8**.

Figs. 76–79. Successive stages in which the ectoderm is conceived to have been removed from the right side, to show the entoderm cells.

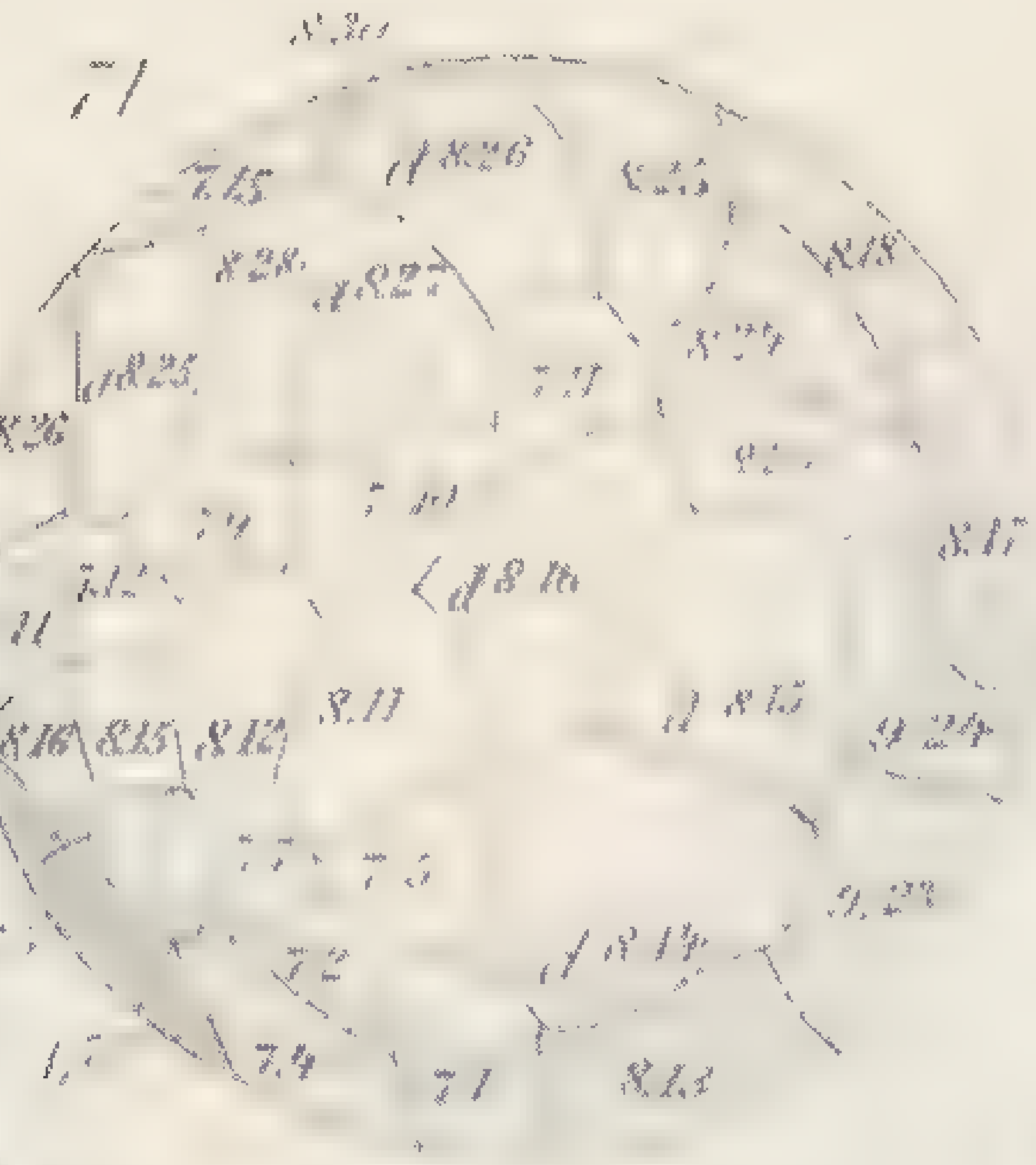
Fig. 76. Egg at the stage shown in Figure 75. A frontal section of this egg is given in Plate **10**, Figure 81.

Fig. 77. Slightly older stage than Figure 76, viewed in the same way.

Fig. 78. Slightly older stage than Figure 77, showing the change in the position of the cells of the entoderm and of those at the animal pole. A view of this egg from the animal pole is shown in Plate **10**, Figure 82.

Fig. 79. Later stage than Figure 78. The entoderm cells have changed position still further, and are approaching cleavage.

71



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7.15
d 8.26
8.25
8.18
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d 8.27
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d 8.25
8.26
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7.10
8.17
7.12
d 8.16
7.11
8.16
8.15
8.12
8.11
d 8.15
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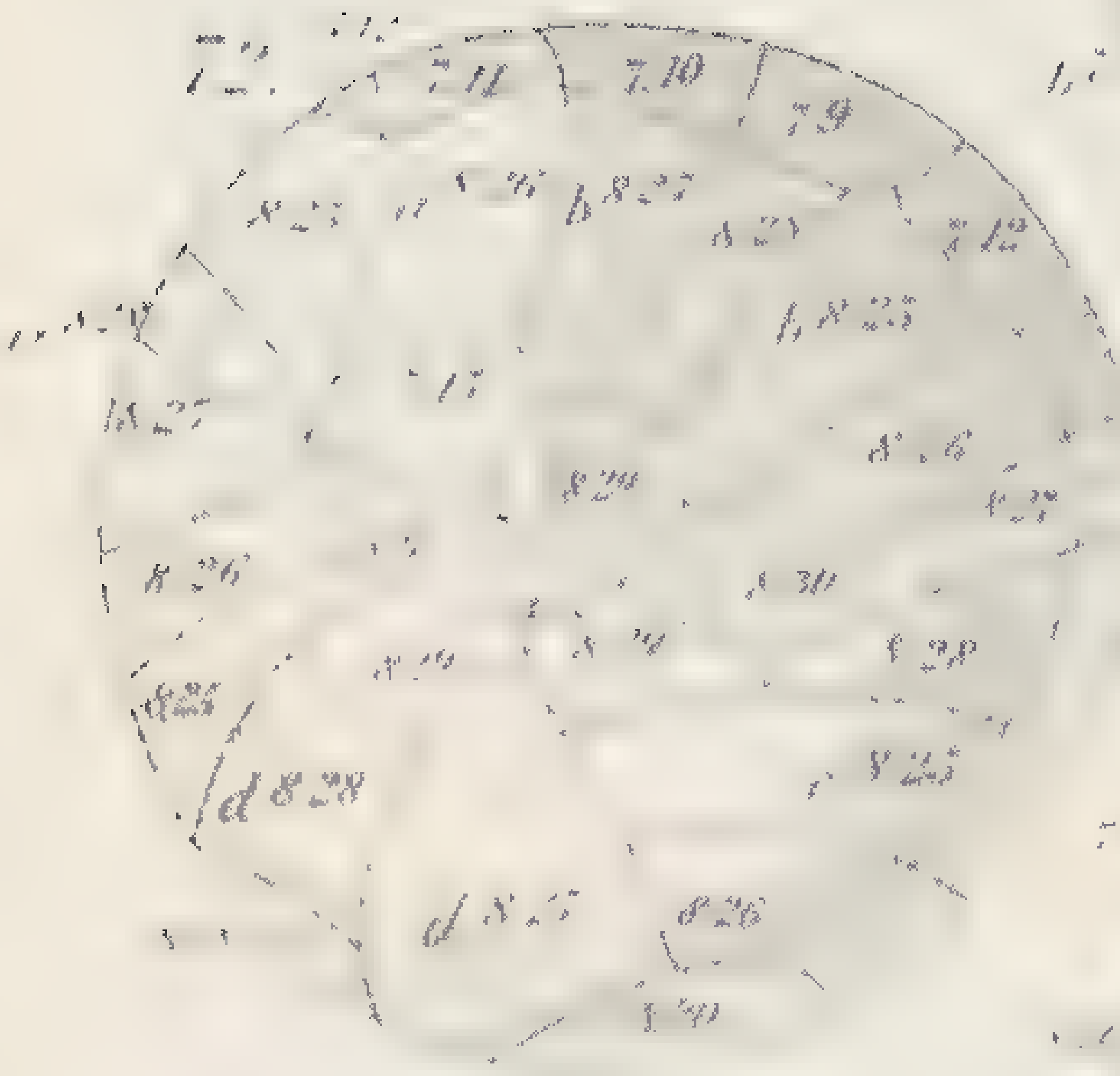
7.2
d 8.14
7.4
7.1
8.13

72.



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7.10
7.9
8.23
d 8.26
b 8.27
8.24
8.12
b 8.23
8.27
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8.29
8.16
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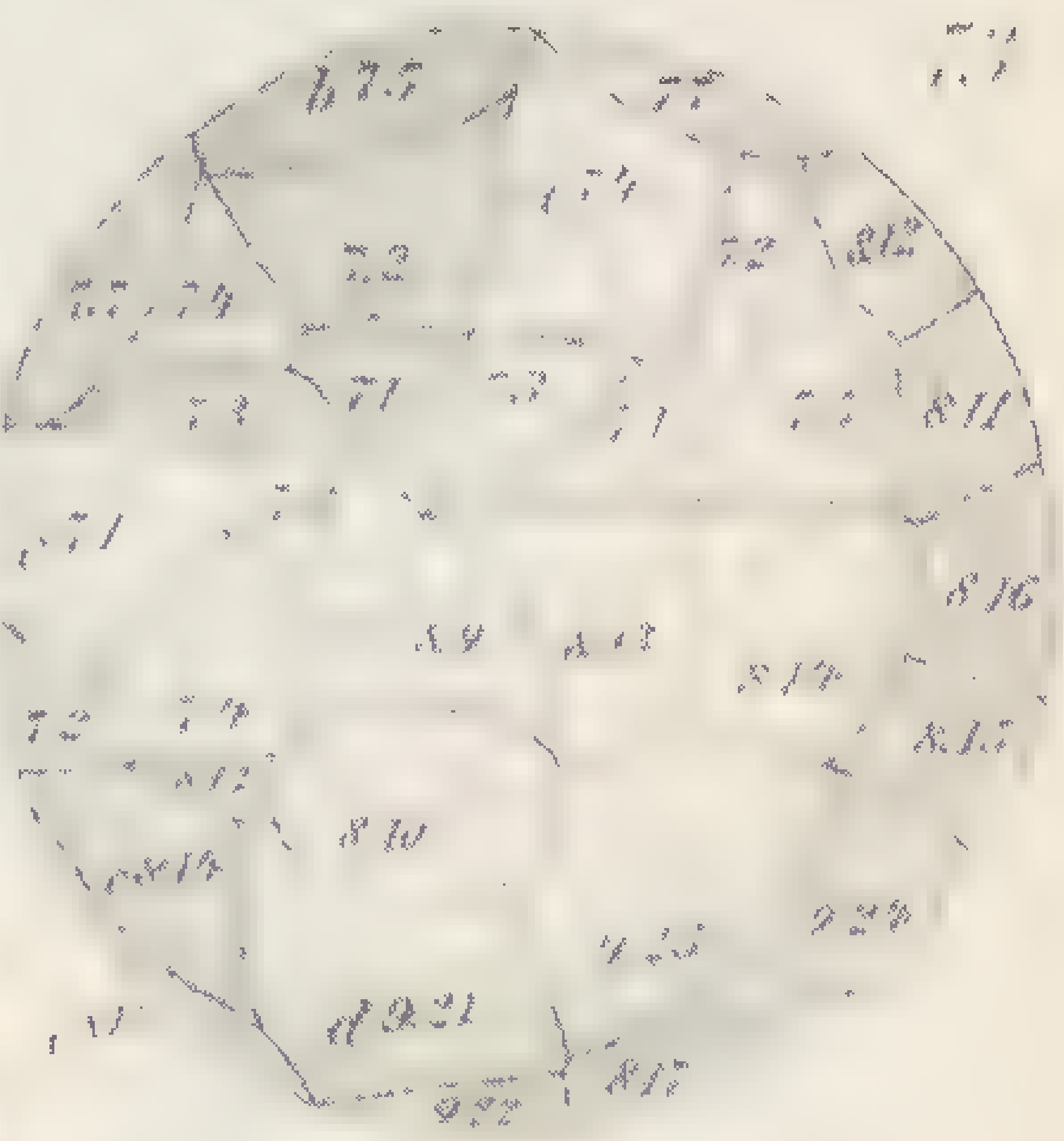
8.30
8.28
8.25
d 8.28
d 8.25
8.26

73



b 7.5
7.7
7.4
7.2
8.12
7.3
7.1
8.11
8.16
8.9
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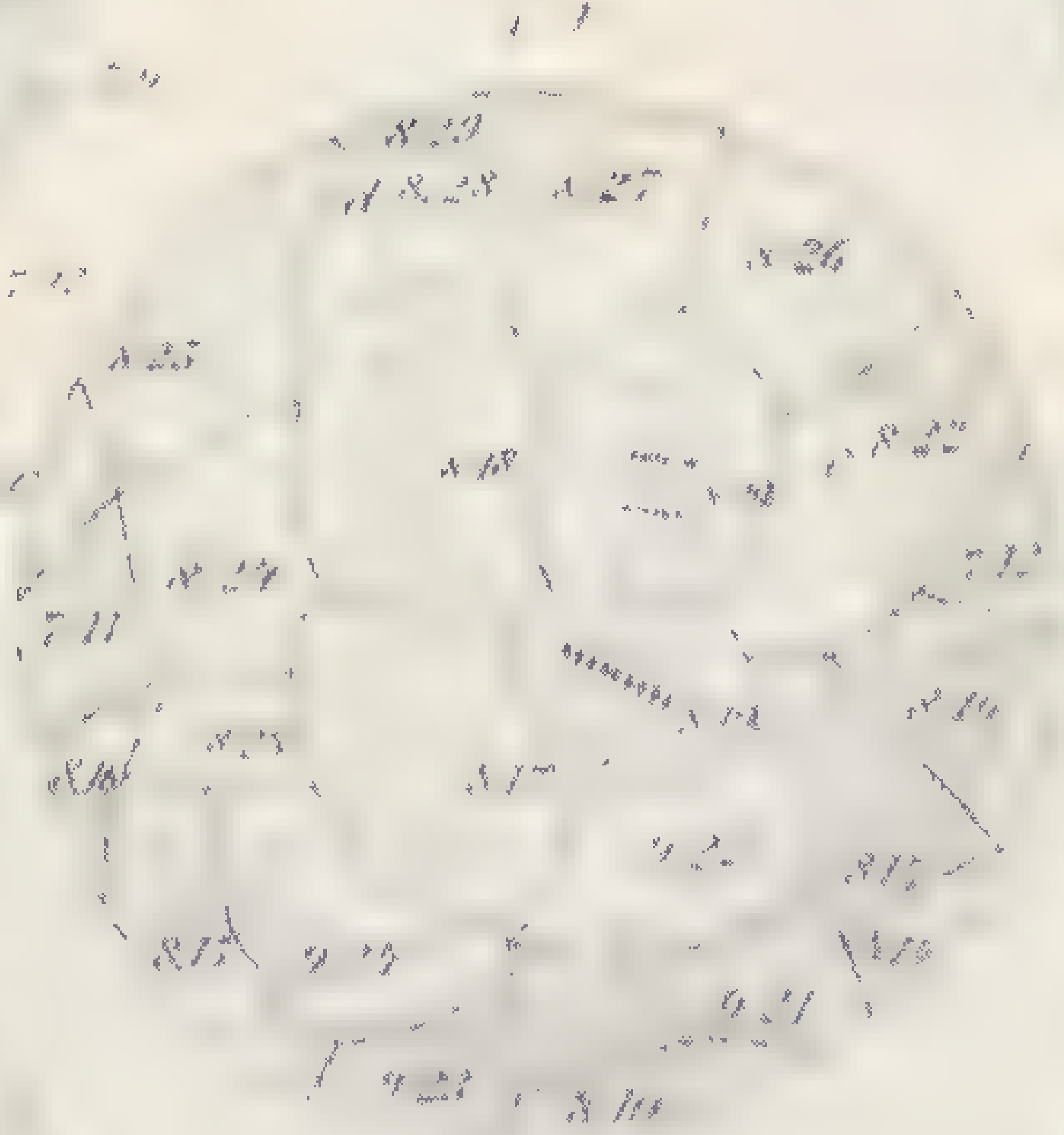
8.15
7.2
7.4
8.12
8.10
9.24
d 9.21
9.22
8.17

74



8.29
d 8.28
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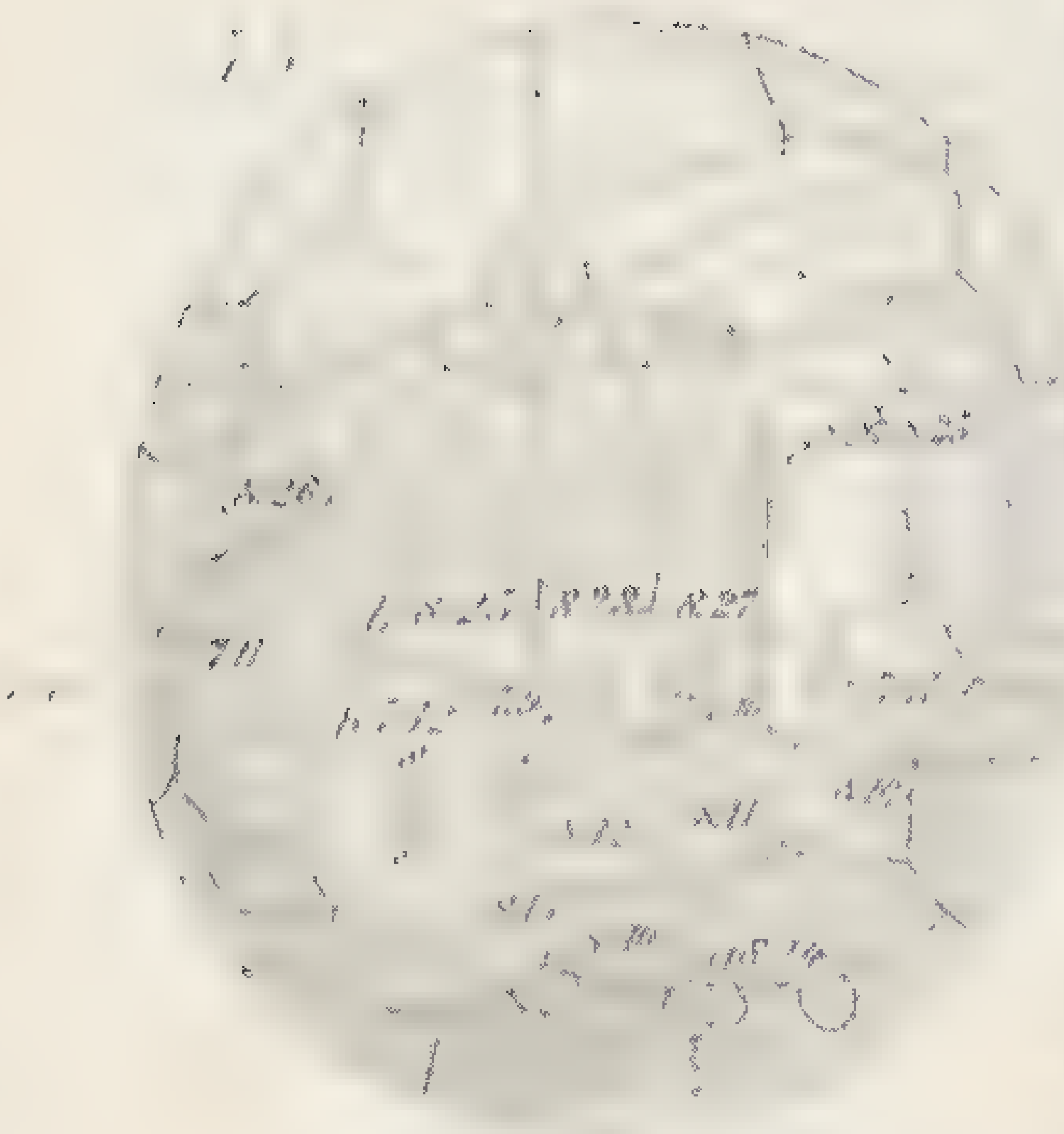
8.10

75



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b 8.23
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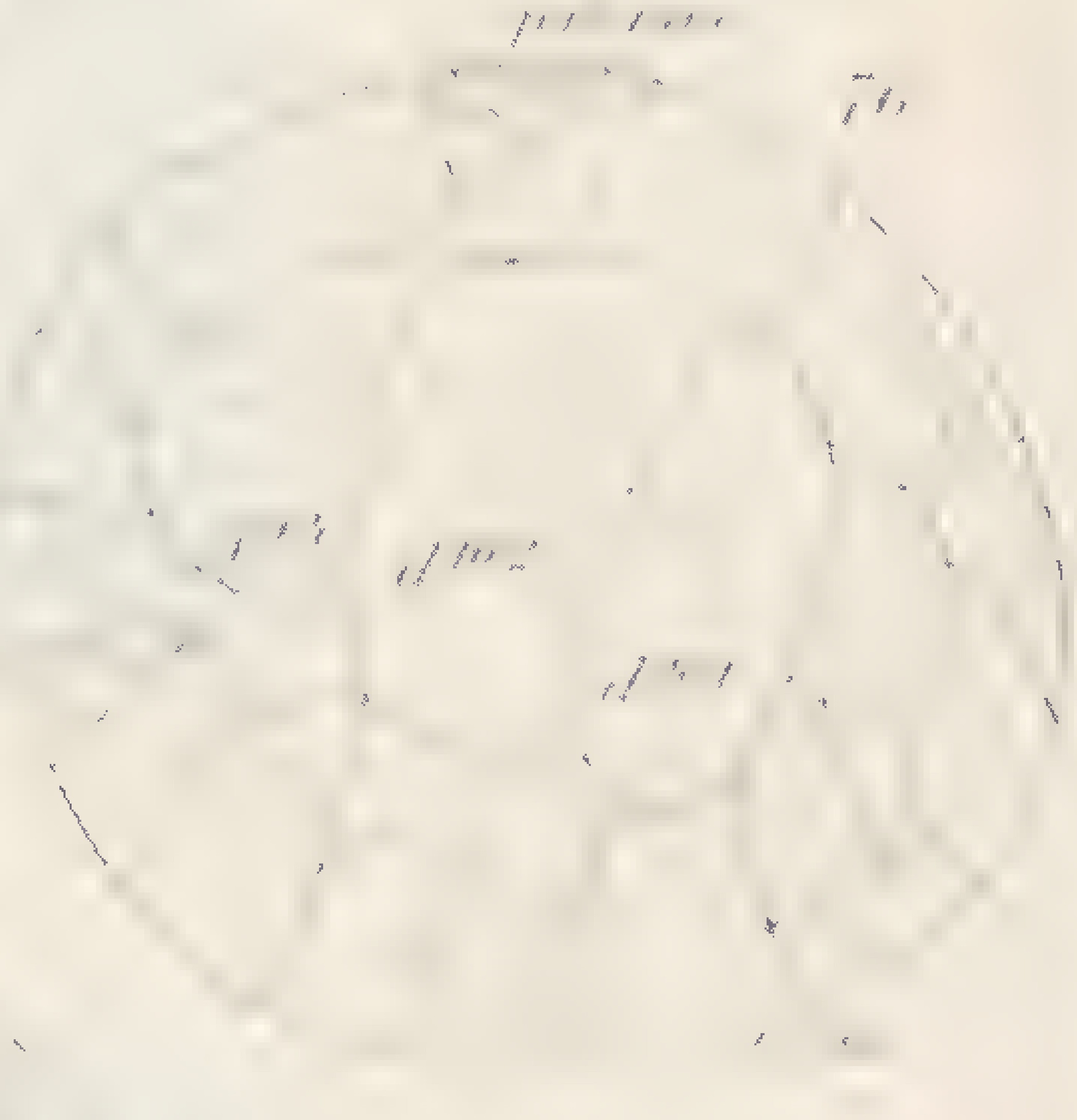
8.11
d 8.14

76



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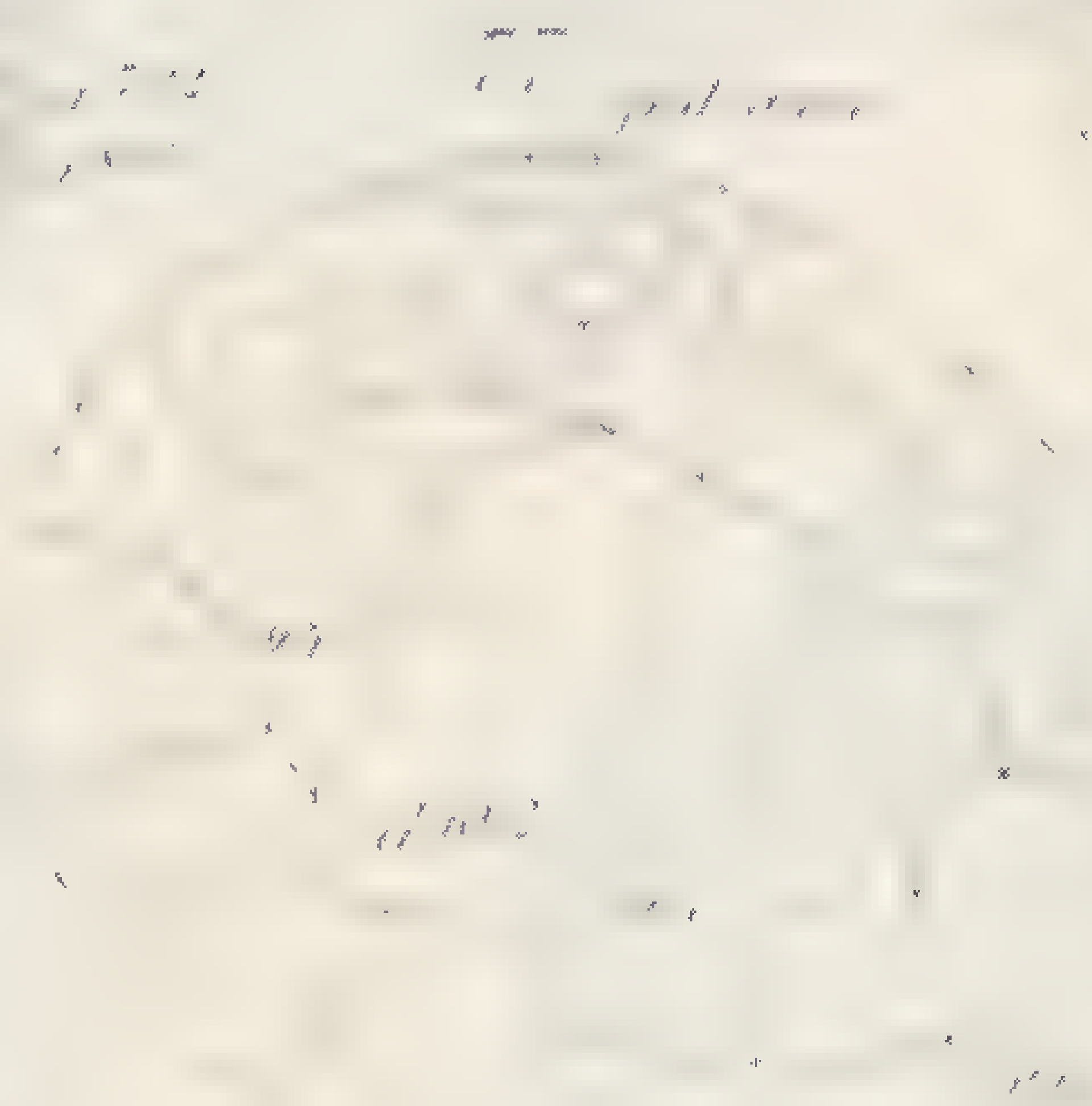


PLATE 10.

Fig. 80. Optical section (approximately frontal) of an egg in the stage seen in Figures 69–74, showing the very unequal division of $d^{8.2}$.

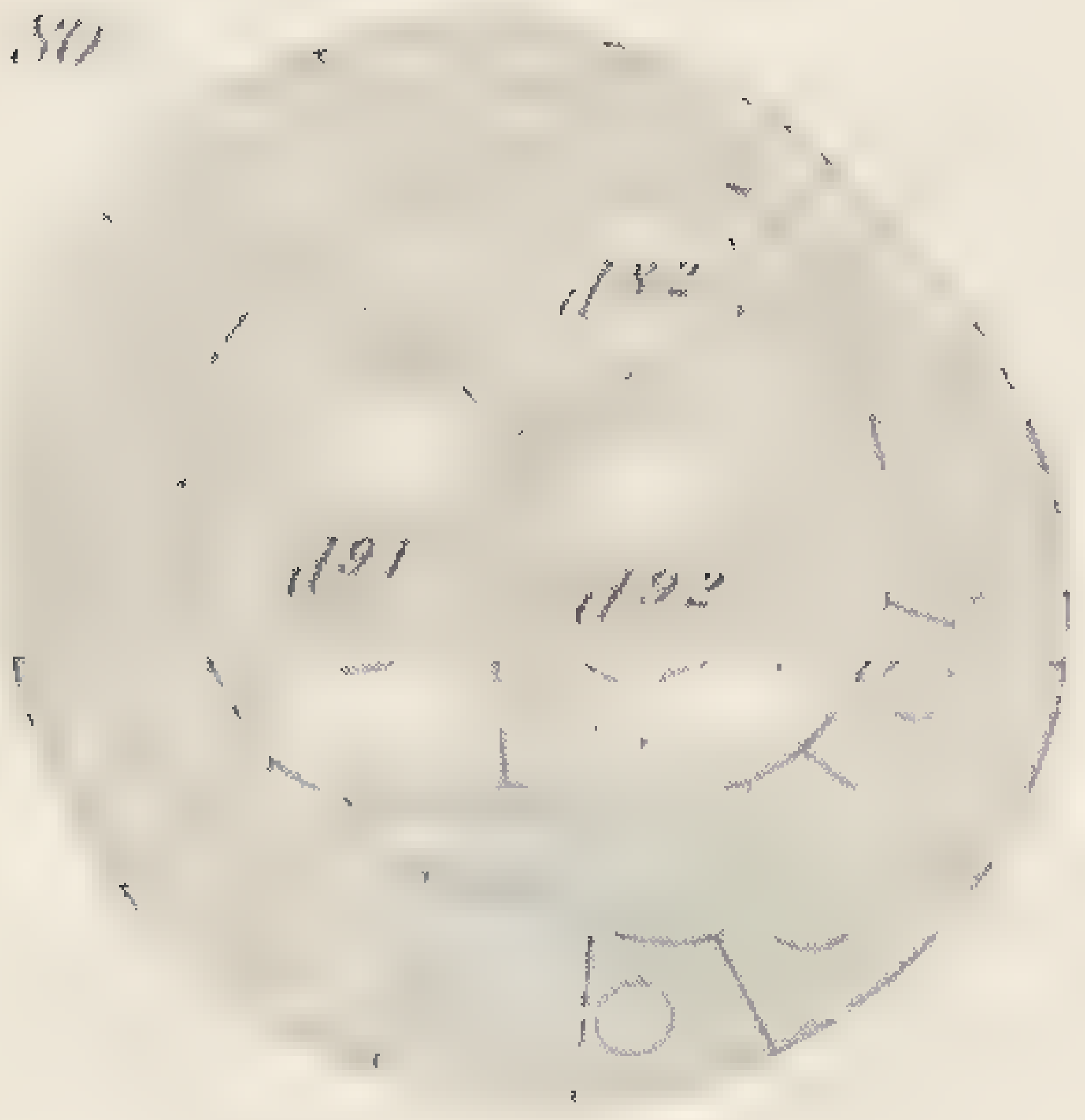
Fig. 81. Optical section (approximately frontal) of a stage later than Figure 80, showing the five large entoderm cells. A side view of the same egg is seen in Plate 9, Figure 76.

Fig. 82. Stage slightly later than that of Figure 81. The ectoderm is supposed to have been removed from the dorso-anterior part of the egg, disclosing the position of the entoderm cells. A side view of the same egg is shown in Plate 9, Figure 78.

Fig. 83. Later stage than the preceding, viewed in the same manner. A spindle has appeared in each of the five large entoderm cells.

Fig. 84. Optical sagittal section of an embryo at about the time of the beginning of the formation of organs.

80.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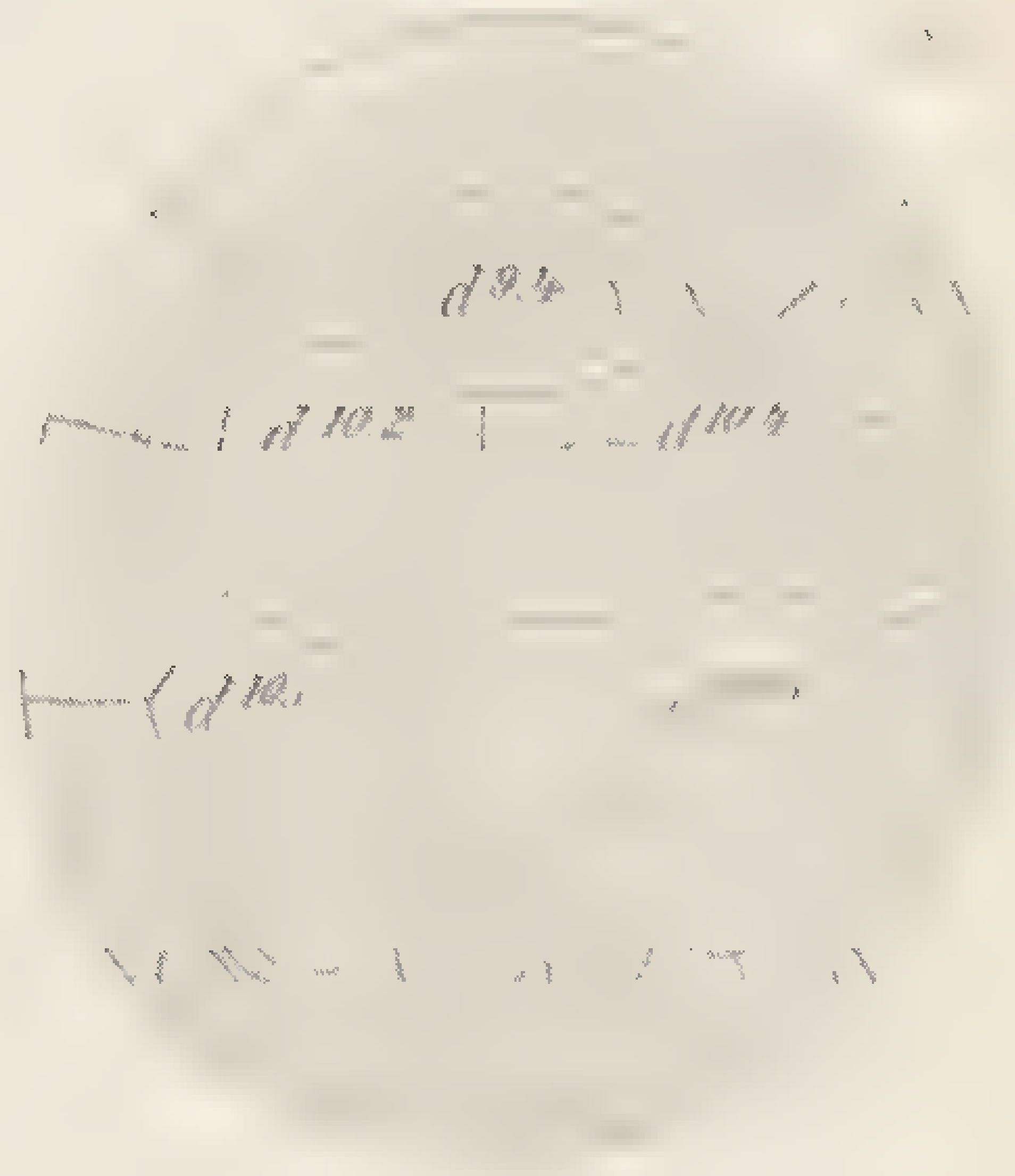
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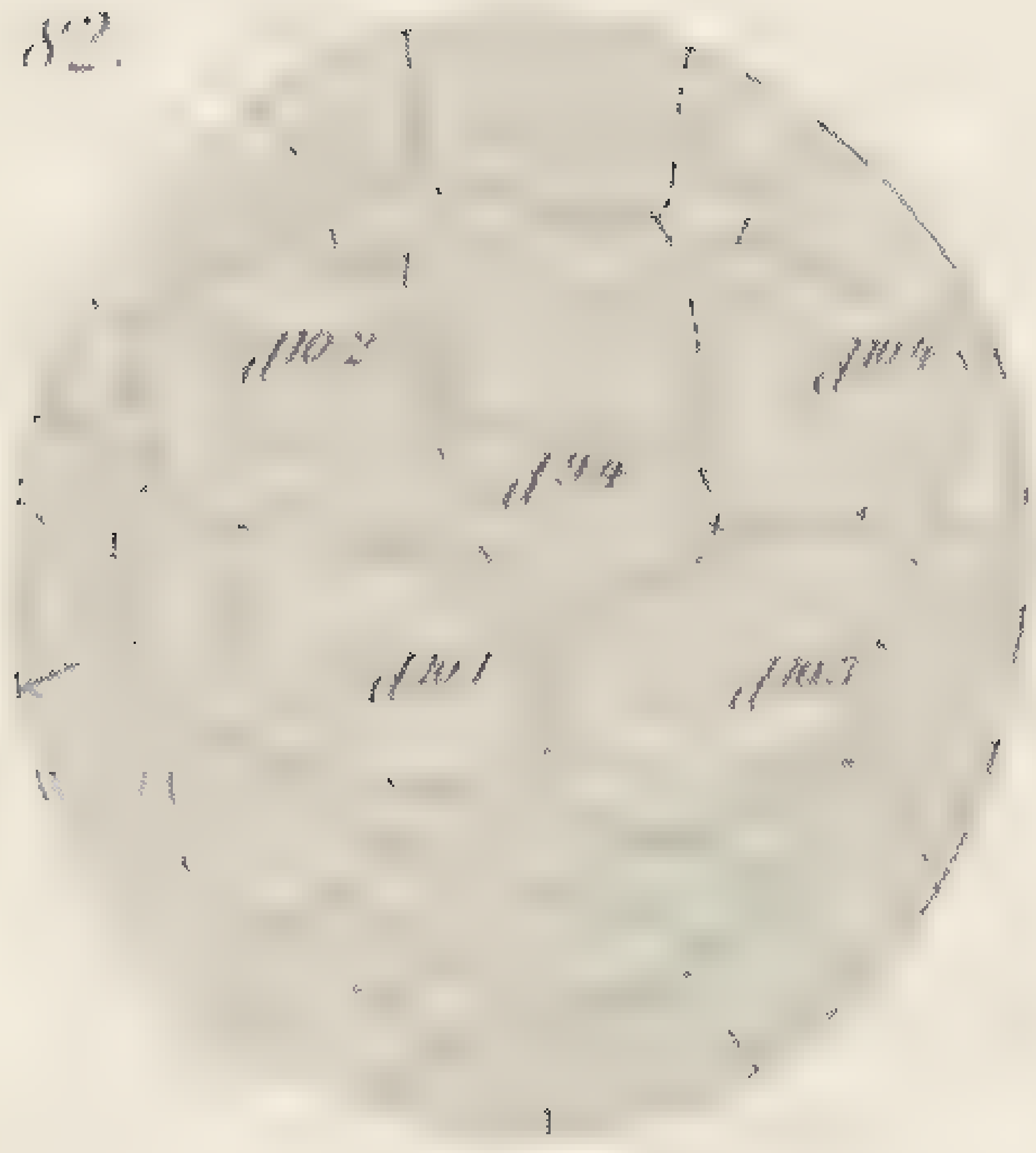
84

82.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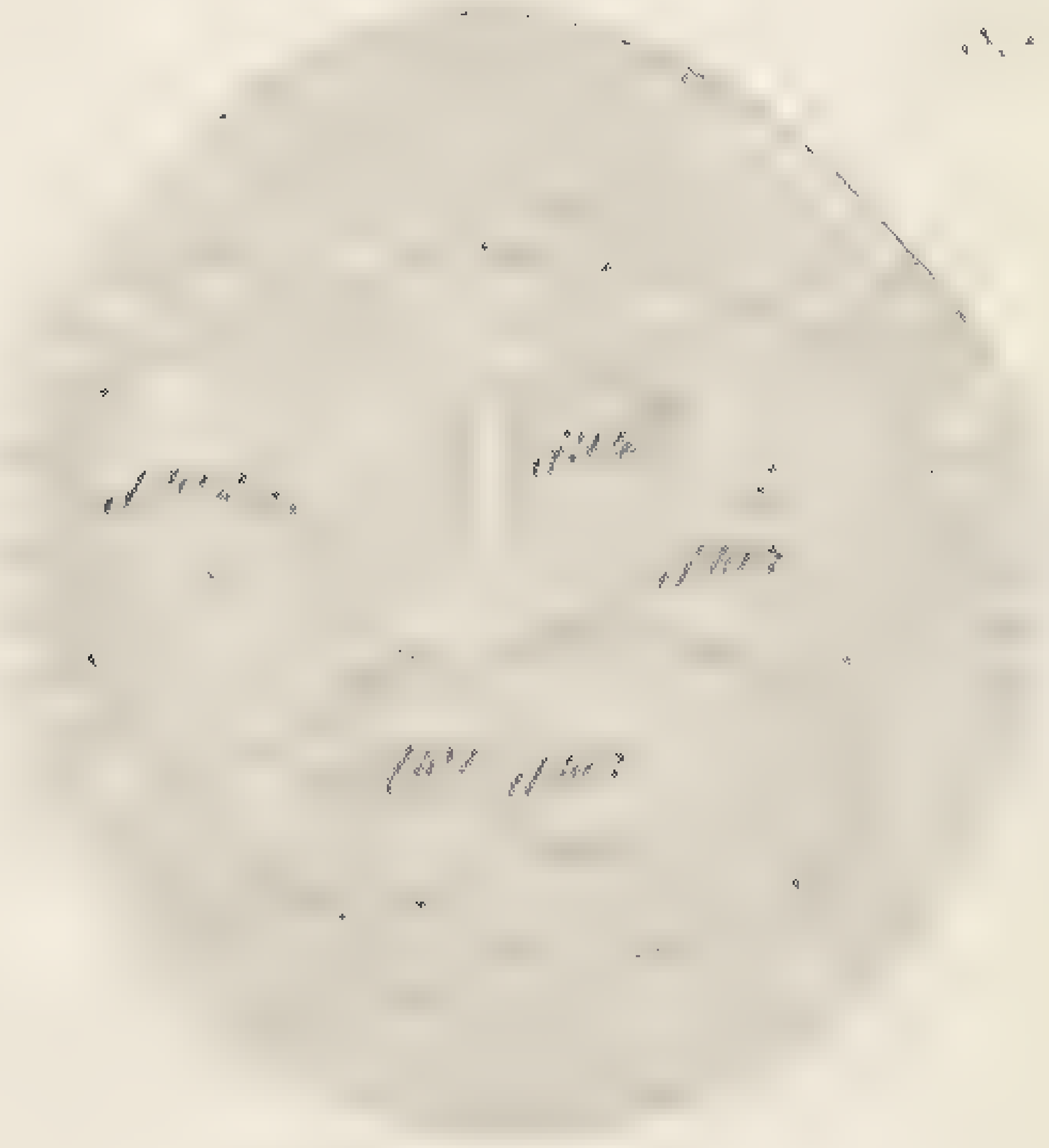
d102
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AT HARVARD COLLEGE.
VOL. XXX. No. 2.

STUDIES FROM THE NEWPORT MARINE LABORATORY. COMMUNICATED BY ALEXANDER AGASSIZ.

No. XL.

SOME VARIATIONS IN THE GENUS EUCOPE.

BY ALEXANDER AGASSIZ AND W. McM. WOODWORTH.

WITH NINE PLATES.

CAMBRIDGE, MASS., U. S. A.:
PRINTED FOR THE MUSEUM.
NOVEMBER, 1896.

No. 2. — *Studies from the Newport Marine Laboratory.*
Communicated by Alexander Agassiz.

XL.

Some Variations in the Genus Eucope. By Alexander Agassiz and W. McM. Woodworth.

We examined for various points nearly four thousand specimens of Eucope (3,917).

Among these we found nine specimens with only three radial canals, twenty with five, and three with six radial canals.

There were fourteen specimens in which one of the radiating canals forked, the forking distal or proximal to the genitals being nearly equally divided.

No less than thirty-nine specimens showed distinct traces of serrations or spurs from one or more of the radial canals.

In eight specimens the radial serrations or spurs were not well defined, and the position and number of the radial canals were indistinct.

In eight specimens marginal tentacles were observed, which had become united at the circular canal, sometimes with the tentacle next to the tentacle with the otolith.

In six specimens there were marked spurs projecting from the base of some of the marginal tentacles.

In eight specimens there were two otoliths in each sense capsule. In four there were three.

In the other specimens the principal variations extended only to the degree of development of the cycles of the marginal tentacles and of the genital organs. The latter showed in some cases peculiar leaf-like expansions extending laterally from the radial canals.

The radial canals were four in number in an overwhelming majority of the specimens examined.

The study of some of the variations in the genus Eucope was undertaken with a view of calling attention to the changes undergone in a species of jellyfish, of which great numbers are always easily obtained during the summer months. The size of the full grown Eucope is so small that with an ordinary hand lens striking variations can at once be detected, and it is possible with a low power to pass in review with comparative ease a large number of specimens.

We hope also to call the attention of zoölogists to the advantages of photography, not only in an investigation of this kind, but also to its application for ordinary purposes of delineation. (See Plate VI. Figs. 3–6.)

Dr. Woodworth photographed the specimens reproduced here on Plates I.–VI., and he has written a short account of the methods he followed.

In reviewing the variations we have observed in one species of Eucope (E. diaphana), we may call attention to the similarity of these variations which occur in this simple Medusa to structures found sometimes in closely allied genera or families, and even in some cases to characters of groups considered as only distantly related to the genus we have examined.

The great number of marginal tentacles in Eucope they have in common with the Æquoridæ.

Eucope shares with the Oceanidæ the limited number of marginal tentacles connected with sense organs. Pendant leaf-like expansions of the genital organs recall those of Melicertidæ.

The presence in Eucope of spurs at the base of the marginal tentacles recalls similar structures in Zygodactyla, Halopsis, and the like.

The forking or branching of the radial canals below the genitals is found also in Willia and in the Berenicidæ, a family allied to the Æquoridæ; the forking is symmetrical in the latter, and asymmetrical in the former genus.

The increase in number of the radial canals from the pouch at the base of the manubrium is a structural feature which is characteristic of the Æquoridæ.

The serration of the radial canals is a generic character of Saphenia and allied genera.

The branching or sending off spurs from the radial canals of Eucope is a structural feature found in Gonionemus, Ptychogena, Polyorchis, and allied forms. The lateral offshoots in Polyorchis being, however, arranged in regular succession on each side of the radial canal, much like the rounds of a ladder.

The anastomosing of the radial canals is a feature now characteristic of the Discophores.

In the increase and special arrangement of the otoliths in the sense organ of Eucope we find the first trace of the specialization of the sense organs of such genera as Oceania, Tiaropsis, and the like.

There were no variations noted in the shape of the digestive cavity, or in the number of actinal lobes of the manubrium,[1] even in specimens with five or six radial canals in place of the normal number (four) o radial canals. The actinal folds were always found to be four in number. In one case only have we found the radiating canal originating from the circular canal. (Plate VIII. Fig. 19.)

The origin of the peculiar club-shaped intertentacular appendages characteristic of Halopsis and Laodicea, as well as the spur at the base of the tentacles in many Æquoridæ, may be referred to the spur-like appendages of the marginal tentacles of Eucope figured in Plate VIII. Figs. 4–13.

And it may not be far out of the way to look upon the coalescence of adjoining marginal tentacles with sense organs as the first indication of such structural features as the radial marginal tentacles of Eucheilota, or even of Boungainvillia, Margelis, or Nemopsis.

It is interesting to note that in Echinoderms there are five radial canals, and four or six or more are considered monstrosities, while in Acalephs four or its multiples are the normal number of radial canals, and five or less are variations.

The specimens of Eucope showing numerical or structural variations were as a rule fully developed males or females, the eggs and spermatozoa being apparently in a healthy condition.

It would be an interesting study in heredity were it possible to breed the variations in Eucope here enumerated, and ascertain how far the structural characters acquired in the variations we have observed can be transmitted, and lead perhaps finally to the formation of types which we have been accustomed to look upon as having no structural relation with the genus.

But it is also possible that in a comparatively simple genus like Eucope these variations are not necessarily to be considered as hereditary; they may indicate possibilities in mechanical combinations which have become characteristic of jellyfishes, and result from their mode of life and the simplicity of their structure. Their repetition in closely allied genera may denote structural affinity, while in distantly allied groups it may be the result of mechanical combinations, and in no way indicate any affinity.

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[1] Variations in the manubrium have been observed in Tubularian Hydroids, such as Lizzia, Dysmorphosa, Hybocodon, Dipurena, and Sarsia; but as they are usually connected with phenomena of reproduction and of budding they have only a distant connection with the line of the present investigation. See an interesting paper by Hartlaub on the reproduction of the manubrium of Sarsia, in Verhandl. d. Deutschen Zool. Gesell., 1896.

The four primary segments of Eucope are of uniform size in the majority of the specimens examined. Whenever there is suppression of a radial canal, as in the case of specimens with three radial canals, the segments are sometimes uniform (Plate III. Figs. 3, 6), or one of the segments, as in Plate III. Fig. 1, is nearly 180°, indicating the total suppression of the fourth radial canal at its normal point of development. See also Plate III. Fig. 4, in which one of the segments is smaller than the two from which the fourth section has been cut.

The inequalities which exist in the segments of some of the specimens can best be expressed by a table: —

$$1;\ 1;\ 1;—\frac{2}{1},\ 1;\ 1;—1;\ \frac{3}{1},\ \frac{3}{1};—1;\ \frac{6}{1},\ \frac{2.5}{1};$$

$$1;\ 1;\ 1;\ 1;—1;\ \frac{5}{1},\ 1;\ \frac{5}{1};—1;\ \frac{3}{1},\ 1;\ \frac{3}{1};—1;\ \frac{4}{1},\ 1;\ \frac{4}{1};—1;\ \frac{2}{1},\ \frac{3}{1},\ \frac{3}{1}$$

$$1;\ 1;\ 1;\ 1;\ 1;—\overbrace{1;\ \frac{2}{1}},\ \frac{3}{1},\ \frac{3}{1},\ \frac{3}{1};—\overbrace{1;\ 1};\ 1;\ 1;\ 1;$$

$$\overbrace{1;\ \frac{1.5}{1}};\ \frac{2.5}{1},\ \frac{2.5}{1};\ \frac{2.5}{1};—\overbrace{1;\ \frac{2.5}{1}};\ \frac{3.5}{1};\ \frac{3.5}{1},\ \frac{3.5}{1};—\overbrace{1;\ \frac{3.5}{1}};\ \frac{4}{1},\ \frac{4}{1},\ \frac{3.5}{1};$$

$$\overbrace{1;\ 1};\ \frac{2}{1},\ \frac{2}{1},\ \frac{2}{1};—1;\ \frac{2}{1};\ 1;\ 1;\ \frac{2}{1};$$

The $\overbrace{\quad}$ indicates a fork of the radial canal.

$$1;\ \frac{2.5}{1};\ 1;\ \frac{2}{1},\ \frac{2}{1},\ \frac{2}{1},—1;\ \frac{2}{1};\ 1;\ \frac{1.5}{1},\ \frac{2}{1},\ \frac{1.5}{1};—1;\ \frac{1.5}{1},\ \frac{2}{1},\ \frac{2}{1},\ \frac{2}{1},\ \frac{2}{1},$$

$$1;\ \frac{1.5}{1},\ \frac{1.5}{1},\ \frac{1.7}{1},\ \frac{1.5}{1},\ \frac{1.5}{1};$$

In this table 1 expresses the smallest segment; the approximate dimensions of the others are represented by multiples of it thus: $\frac{2}{1}$ indicates that a segment is twice as wide at the periphery as the smallest; $\frac{3.5}{1}$, that it is three and a half times as wide. Of course, the value of 1 is a different value in each case.

1; 1; 1; is the formula for the segments of a Eucope in which they are of equal size, the radial canals forming an angle of 120° at the centre.

1; 1; 1; 1; and 1; 1; 1; 1; 1; would each denote the formula for segments of equal dimensions in a Eucope with four and one with five segments.

The formation of additional radial tubes may be due to the growth of independent tubes from the pouch at the base of the digestive cavity, or from the forking of tubes, the new canals eventually reaching the marginal canal. In one case we observed a radial canal which had its origin at the periphery and did not extend to the base of the manubrium (Plate VIII. Fig. 19). Such a formation of a new radial canal from the circular canal suggests a similar structure in the short canals, in which are found clusters of lasso cells extending at right angles from the periphery between the primary radial canals of Willia, and perhaps other Medusæ, in which we have clusters of lasso cells extending a short distance on the outer surface of the umbrella from the marginal canal.

In the great majority of the specimens of Eucope observed, the radial canals are tubes with walls nearly parallel all the way from the base of the digestive cavity to the marginal canal (Plates I.–VI.). But in a great many instances this parallellism does not exist, and we find on the edge of some of the radial canals slight serrations, as in Plate VII. Figs. 1–4 and 6. These serrations vary greatly in size, and in some cases become short spurs (Plate II. Fig. 4, Plate III. Fig. 3, Plate VII. Figs. 2, 3, 5, 7), or even spurs of considerable length (Plate VII. Figs. 6, 9, 10); the longer spurs becoming often the forks of the primary radial canals (Plate III. Figs. 1, 2–5, Plate VI. Figs. 1, 2, Plate VII. Fig. 5), either above or below the genital pouches. Or the spurs may form connecting canals between the radial tubes (Plate VII. Fig. 4, Plate VIII. Fig. 20), or a rudimentary circular canal round the base of the manubrium (Plate VII. Fig. 8).

Starting with the normal state, in which the genitals are equally developed, we find five or six variations, which cover by far the greater number of the specimens examined.

The greatest number of the specimens (622 out of 1146) examined for variations in the genital organs were normal, the four genitals being equally developed; the females were more numerous than the males; of the latter there were 175, and of the former 447. This stage is represented in the table by 1; 1; 1; 1; in which 1 means that the genitals are fully developed, and equally so on each radial canal, of which there are four.

The next stage represented by the formula, 1; 1; 1; 0; the genitals were atrophied on one of the radial canals and equally developed on the others. Seventy-eight specimens of this stage, 49 females and 29 males.

The next most frequent stage is that in which two adjoining genitals are fully developed; the others are of the same size, but less well developed; that stage is represented by the formula 1; 1; 2; 2; Out of the 1,146 specimens examined there were only 74 specimens of this stage, of which 45 were females and 29 males.

Next comes the stage in which only one of the genitals is fully developed; the others are less so, corresponding to the formula 1; 2; 2; 2; 39 females and 27 males.

In the order of frequency of occurrence comes:

Eucope with the formula 1; 1; 1; 2; — 28 females and 17 males.

Then comes the stage in which the genitals were unequally developed: 20 females and 21 males.

Next,

Eucope with the formula 1; 0; 0; 0; — 24 females and 13 males.
" " " 1; 2; 0; 0; — 15 " " 3 "
" " " 1; 2; 2; 0; — 10 " " 8 "

In specimens with three radial canals we observed only one specimen in which the genitals were uniformly developed. On Plate III. are seen (Fig. 1) a specimen in which one of the canals forks at the extremities of the heart-shaped genitals, forming three primary segments of nearly equal size extending to the centre of the disk, with a small sector cut from the outer edge of two adjoining segments.

Figure 4 of the same plate shows a specimen with three radial canals and four genital pouches, but the canal which forks subdivides above the genitals so near the centre of the disk as to subdivide the disk into four nearly equal segments.

A variation similar to that of Plate III. Fig. 1, for a three-rayed Eucope, has been observed in a four-rayed Eucope (Plate III. Fig. 2), in which the fifth sector is a comparatively small triangle cut out from the periphery of two of the adjoining sections, extending to the centre of the disk.

In Figure 5 of Plate III. the forking of the four-rayed Eucope, taking place nearer the centre of the disk, subdivides the disk into segments of more uniform size, and it closely resembles a five-rayed Eucope.

In five-rayed Eucope only one specimen was observed with equally developed genitals (Plate V. Fig. 1). Those figured on Plate VI., as well as the others on Plates IV. and V., all show a very great range of variation in their development. In two cases (Plate IV. Figs. 3, 6) two of the genitals are wanting.

In Plate VI., Figures 1 and 2 are those of two specimens with five radial canals, both of which fork so near the base of the manubrium as to divide the disk into six nearly equal segments.

The accompanying table will show the other variations which have been observed, and their frequency.

1146 Specimens examined for Variations in the Development of the Genital Organs on each of the Four Radial Canals.

With Three Radial Canals, or Three with Fork.

1; 2; 3;	3	females,	and	1	male.
1; 1; $\underbrace{1; 1;}$	2	"	"	1	"
1; 1; 1;	1	female.			
1; 1; 0;	2	"			

With Four Radial Canals.

1; 1; 1; 1;*	447	females	and	175	males.
1; 1; 1; 0;	49	"	"	29	"
1; 1; 2; 2;	45	"	"	29	"
1; 2; 2; 2;	39	"	"	27	"
1; 1; 1; 2;	28	"	"	17	"
1; 2; 3; 4;	20	"	"	21	"
1; 0; 0, 0;	24	"	"	13	"
1; 1; 0; 0;	21	"	"	9	"
1; 2; 3; 0;	19	"	"	7	"
1; 2; 0; 0;	15	"	"	3	"
1; 2; 2; 0;	10	"	"	8	"
1; 1; 2; 0;	6	"	"	8	"
1; 1; 2; 3;	5	"	"	4	"
1; 2; 2; 3;	5	"	"	4	"
1; 2; 3; 3;	4	"	"	3	"
1; 0; 0; 2;	3	"	"	2	"
1; 0; 2; 3;	3	"	"	2	"
1; 1; 0; 2;	4 males				

[*] 1, fully developed genitals; 2, genital smaller than 1; 3, genital smaller than 2; 4, genital smaller than 3; 5, genital smaller than 4; 0, genital organ absent. The semicolon (;) indicates the position of the radial canal. The $\smile$ indicates the forking of the radial canal.

1; 0; 2; 0;	4 females.
1; 2; 2; 3;	3 "
0; 0; 0; 0;	3 specimens.
1; 2; 0; 3;	2 females.
1; 2; 1; 3;	1 "
1; 2; 3; 1;	1 "
1; 1; 1; 4;	1 "
1; 0, 0; 1;	1 male.
1; 1; 1; 3;	1 female
1; 0; 1; 0;	1 "
1; 1; 4; 4;	1 "
1; 3; 2; 0;	1 "
1; 2; 0; 4;	1 male.
1; 2; 1; 2;	1 female.
1; 2; 2; 4;	1 "
1; 0; 3; 4;	1 "

With Five Radial Canals, or Four and Five with Fork.

$1;\ \underbrace{2;\ 2;}\ 2;\ 0;$	1 female.
1; 2; 3; 4; 5;	2 females and 1 male.
1; 3; 0; 2; 0;	1 "
1; 2; 2; 3; 4;	1 male.
1; 3; 3; 4; 4;	1 female.
1; 2; 2; 2; 3;	1 "
1; 1; 3; 0; 4;	1 "
1; 1; 1; 1; 1;	1 male.
1; 3; 3; 3; 4;	1 "
1; 1; 3; 4; 4;	1 female.
2; 4; 2; 4; 5;	1 "
1; 2; 3; 0; 4;	1 male.
$1;\ 3;\ 3;\ \underbrace{4;\ 4;}$	4 females and 1 male.
$1;\ 1;\ \underbrace{4;\ 4;}\ 2;$	3 females and 1 male.

The variations in the shape of the genitals from a circular to an elliptical outline to lobate, foliate, or elongate shape (Plate VIII. Fig. 21) is usually connected with the growing together of the genitals of adjoining radial canals (Plate VIII. Fig. 18) or with their separation into two distinct bodies when the radial canals fork (Plate III. Figs. 1, 5).

It will be seen that in Eucope, as has been observed in Aurelia, the tendency in the numerical variation of the genitals is greatest in the direction of an increase rather than a diminution in their number. A similar tendency is also noticed in the numerical variation of the radial canals of the otoliths.

The youngest specimens of Eucope observed (eleven in number) had twelve tentacles in all, one at the base of each of the four radial canals and two in each quadrant with a sense organ, and without any tentacles intermediate between the radial and the sense organ tentacle. It is possible that these specimens may belong to a different species, as the youngest specimens of Eucope diaphana I have raised from the hydroid already had 24 tentacles.[1] But of course it is possible that the above stage may have dropped from the reproductive calycle at an earlier stage of development.

Were the marginal tentacles to develop regularly we should have as the third stage a Eucope with 48 tentacles, a fourth stage with 96, and a fifth stage with 192.

But as far as our experience shows, we find normal Eucope only in the first and second stages, and even then there are numerous variations.

The formulæ for the normal stages are, calling T any marginal tentacle,

T_c, the primary tentacle at the base of the radial canals;

T_0, the primary tentacle with a sense organ at its base;

t_1, the first set of marginal tentacles appearing between T_c and T_0 in the stage with 24 marginal tentacles;

t_2, the second set of marginal tentacles on each side of t_1, in the stage with 48 marginal tentacles;

t_3, the third set of marginal tentacles on each side of t_2, in the stage with 96 marginal tentacles;

t_4, the fourth set of marginal tentacles on each side of t_3, in the stage with 192 marginal tentacles;

t_5, the fifth set of marginal tentacles on each side of t_4, in the next stage with as many (theoretical number 31 tentacles) as $16t_5$, $8t_4$, $4t_3$, $2t_2$, $1t_1$ tentacles between the (T_c and T_0) radial canal and sense organ tentacle, or 95 tentacles in each quadrant between the radial canals, or a total theoretical number of 384 marginal tentacles.

The formula of the youngest stage, or first stage, would be

$$\Sigma\ (4)\ T_c + (4)\ 2\ T_0 = 12\ T,$$

or two tentacles with a sense organ in each quadrant.

That of the second stage,

$$\Sigma\ (4)\ T_c + (4)\ 2\ T_0 + (4)\ 3t_1 = 24\ T,$$

or two tentacles with a sense organ in each quadrant with a tentacle of the t_1 order between each and the radial canal and sense organ.

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[1] Proc. Boston Soc. Nat. Hist., June 4, 1862, p. 92.

That of the third stage,

$$\Sigma\ (4)\ T_c + (4)\ 2\ T_0 + (4)\ 3t_1 + (4)\ 6t_2 = 48\ T,$$

the same as the second stage, with an additional tentacle of the t_2 order intercalated on each side of the t_1 tentacles, making 11 tentacles in each quadrant, or three between each of the three primary divisions of the quarter.

The fourth stage,

$$\Sigma\ (4)\ T_c + (4)\ 2\ T_0 + (4)\ 3t_1 + (4)\ 6t_2 + (4)\ 12t_3 = 96\ T,$$

or a tentacle of the third order, t_3, intercalated on each side of t_2, thus making 7 tentacles in each of the primary divisions of the quarter.

The fifth stage, with 192 tentacles, would have for its formula,

$$\Sigma\ (4)\ T_c + (4)\ 2\ T_0 + (4)\ 3t_1 + (4)\ 6t_2 + (4)\ 12t_3 + (4)\ 24t_4 = 192\ T,$$

or a tentacle of the fourth order, t_4, intercalated on each side of t_3, thus making 15 tentacles in each of the primary divisions of the quarter.

The theoretical formula for the sixth stage, with 384 tentacles, would be,

$$\Sigma\ (4)\ T_c + (4)\ 2\ T_0 + (4)\ 3t_1 + (4)\ 6t_2 + (4)\ 12t_3 + (4)\ 24t_4 + (4)\ 48t_5 = 384\ T,$$

a tentacle of the fifth order, t_5, having been intercalated on each side of the tentacles of the t_5 order, thus making 41 tentacles in each of the primary divisions of the quarter.

It is not necessary to carry these stages any further, as the largest number of tentacles observed in any primary division of each quadrant is not greater than thirteen, which would limit it to a modified stage of 192 marginal tentacles.

The accompanying tables have been arranged so as to indicate the association, whatever their number may be, with the highest number of marginal tentacles occurring in any primary quadrant. This number may be a normal number between any T_c and T_0, or between any T_0 and T_0, and may be due to the absence of one of the T_0 tentacles, or of both, in the quadrant.

A glance at the tables shows how few of the specimens examined possessed the normal number of tentacles. We should have as the normal stages of each quadrant for the four stages up to 192 tentacles.

T_c, T_0, T_0, T_c, first stage, with 12 marginal tentacles.

T_c, t_1, T_0, t_1, T_0, t_1, T_c, second stage, with 24 marginal tentacles; or, as in the tables, 1, 1, 1; 1, 1, 1; 1, 1, 1; 1, 1, 1; of which eleven specimens were seen from the number tabulated for numerical variations.

The formula for the third stage, with 48 tentacles, is,

$$\mathrm{T}_c,\ t_2,\ t_1,\ t_2,\ \mathrm{T}_0,\ t_2,\ t_1,\ t_2,\ \mathrm{T}_0,\ t_2,\ t_1,\ t_2,\ \mathrm{T}_c,$$

or, as in the table, 3, 3, 3; 3, 3, 3; 3, 3, 3; 3, 3, 3; of which fourteen specimens were found from the number tabulated for variations.

In the fourth stage the formula is,

$$\mathrm{T}_c,\ t_3,\ t_2,\ t_3,\ t_1,\ t_3,\ t_2,\ t_3,\ \mathrm{T}_0,\ t_3,\ t_2,\ t_3,\ t_1,\ t_3,\ t_2,\ t_3,\ \mathrm{T}_0,\ t_3,\ t_2,\ t_3,\ t_1,\ t_3,\ t_2,\ t_3,\ \mathrm{T}_c,$$

or 96 marginal tentacles.

Of this normal stage only two specimens were observed.

Of the fifth stage, with 192 marginal tentacles, of which the following is the formula, not a single normal stage was observed:

$$\mathrm{T}_c,\ t_4,\ t_3,\ t_4,\ t_2,\ t_4,\ t_3,\ t_4,\ t_1,\ t_4,\ t_3,\ t_4,\ t_2,\ t_4,\ t_3,\ t_4,\ \mathrm{T}_0,\ t_4,\ t_3,\ t_4,\ t_2,\ t_4,\ t_3,\ t_4,\ t_1,\ t_4,\ t_3,\ t_4,\ t_2,\ t_4,\ t_3,\ t_4,\ \mathrm{T}_0,\ t_4,\ t_3,\ t_4,\ t_2,\ t_4,\ t_3,\ t_4,\ t_2,\ t_4,\ t_3,\ t_4,\ t_1,\ t_4,\ t_3,\ t_4,\ t_2,\ t_4,\ t_3,\ t_4,\ \mathrm{T}_c.$$

But a few specimens were seen with the normal number of 15 tentacles in some of the primary divisions of one quadrant.

Of the sixth stage, with 41 tentacles in each of the primary divisions of the quadrant, not a single specimen was collected.

It is interesting to note that in the specimens which may be said to belong to the second stage, some of the primary divisions of the quadrants remain in the first stage with only one tentacle, and others with two, the third tentacle not having developed.

Between the second and third stage, in a number of specimens, the greatest number of tentacles in a primary division of the quadrant is four, and the smallest one. In a number of cases two or even three of the quadrants remain in the second stage, and only in one quadrant do we find four tentacles in a primary quadrantic division. Similarly, we find a number of specimens with five tentacles as the largest number in any primary quadrantic division, and some of the quadrants in the second stage, but none in the first. Even when we come to six tentacles as the largest number of tentacles in any primary quadrantic division, we still find some of the primary quadrantic divisions in the second stage, and occasionally a whole quadrant or a quadrantic division above the first stage, as is the case in the second stage and stages intermediate with the third.

Only four specimens typical of the normal third stage were observed, and the great majority of the other specimens in which seven tentacles were found in a quadrantic division belonged to stages approximating the third more nearly than the second. Only a small proportion of the specimens were observed in which the quadrantic divisions belonged to the second stage. As will be noticed from the tables, by far the largest number of specimens examined belonged in or near the category of the third stage. The number of specimens with more than seven tentacles in a primary quadrantic division became less as the number of tentacles increased, and the whole number of tentacles in any quadrant was also more variable.

No fully developed Eucope was found in the fourth stage with 15 tentacles in the primary divisions of all the quadrants (see table for 15 tentacles, page 136), and when we come to a larger number of tentacles for each primary quadrantic division the irregularities of the marginal tentacles leave us in doubt in which stage to place them.

In the more advanced stages sometimes only one quadrant is affected, the others remaining less advanced. This seems to indicate that the mere multiplication (of parts) of the marginal tentacles beyond the third stage is accompanied with greater and greater irregularities in the different quadrants as the number of marginal tentacles increases.

A special table has been made to show the variations observed from the normal number of eight otoliths and of the four radial canals.

In the tables the notation 2, 3, 3 ; 3, 3, 3 ; 3, 3, 3 ; 3, 3, 3 ; the 2 indicates that starting from a radial canal there are two marginal tentacles between it and the marginal tentacle with a sense organ indicated by a comma ; the 3 indicates three marginal tentacles between it and the next sense tentacle, and again three marginal tentacles between it and the radial canal ; and so on, the figure always indicating the number of marginal tentacles, the comma the sense-bearing tentacles, and the semicolon the tentacles at the base of the radial canal.

Variations in number of intervening marginal tentacles, or of sense organs, or of radial canals, are thus easily indicated.

Thus,

11, 5 ; 11, 5 ; 5, 5, 5 ; 6, 5, 4 ;

indicates the presence of six sense-bearing tentacles, there being only one in two of the quadrants.

3, 6, 2 ; 3, 3 ; 10, 9, 9 ; 10, 7, 8 ; 8, 7, 12 ;

on the contrary, is a Eucope with five radial canals and nine otoliths, while

4, 6, 5 ; 2, 8, 6, 7 ; 1, 11, 2 ;

indicates a Eucope with only three radial canals and seven otoliths.

In the tables, except in the list marked irregular, those specimens which differ from the normal in the number of their primary quadrantic divisions or quadrants, or in that of the otoliths, or in that of the radial canals, are marked by an asterisk.

First Stage.

With only one tentacle in each primary subdivision of the quadrant.

1, 1, 1; 1, 1, 1; 1, 1, 1; 1, 1, 1; (11).

Second Stage.

With not more than three tentacles in any subdivision: —

3, 3, 3; 3, 3, 3; 3, 3, 3; 3, 3, 3; (11)
*1, 3, 3; 3, 3, 3; 3, 3, 3; 3, 3, 0;
2, 3, 3; 3, 3, 3; 3, 3, 3; 3, 3, 3;
3, 3, 3; 3, 3, 3; 3, 3, 3; 2, 3, 3;
3, 2, 2; 2, 3, 2; 2, 3, 3; 2, 3. 2;
3, 3, 3; 3, 3, 3; 3, 3, 3; 3, 3, 2;
1, 3, 1; 1, 3, 1; 1, 3, 1; 1, 3, 1;
3, 2, 2; 2, 3, 2; 3, 3, 2; 2, 3, 1;

With not more than four tentacles in any subdivision: —

3, 3, 3; 4, 3, 4; 3, 3, 4; 3, 3, 3;
3, 3, 3; 4, 4, 3; 3, 3, 3; 3, 3, 3;
2, 3, 1; 3, 3, 4; 3, 3, 3; 4, 3, 2;
3, 3, 3; 3, 3, 3; 3, 3, 3; 3, 4, 3;
3, 3, 4; 4, 4, 4; 4, 3, 4; 4, 4, 4;
3, 3, 3; 3, 3, 3; 3, 4, 3; 3, 3, 4;
3, 3, 4; 4, 3, 3; 3, 3, 4; 4, 3, 3;
3, 3, 3; 3, 3, 3; 3, 3, 3; 3, 3, 4;
3, 3, 3; 3, 3, 3; 3, 3, 3; 4, 3, 3;
3, 3, 3; 3, 3, 3; 3, 3, 4; 3, 2, 4;
3, 3, 3; 3, 3, 4; 4, 4, 4; 3, 4, 4;
3, 3, 3; 3, 3, 4; 4, 3, 3; 4, 3, 3;
3, 3, 3; 4, 4, 3; 4, 4, 4; 4, 4, 3;
3, 3, 3; 3, 3, 4; 3, 3, 3; 3, 4, 3;
3, 3, 3; 4, 3, 3; 3, 3, 3; 4, 3, 3;
3, 3, 3; 3, 3, 3; 3, 3, 3; 4, 3, 4;
3, 3, 3; 3, 3, 3; 3, 3, 4; 3, 4, 3;
3, 3, 3; 1, 3, 3; 3, 2, 3; 1, 4, 2;
3, 3, 3; 3, 3, 3; 4, 3, 3; 4, 3, 4;
3, 3, 3; 3, 3, 3; 3, 3, 3; 3, 3, 4;
*3, 3, 3; 3, 3, 4; 3, 3, 3; 4, 4, 4, 1;
4, 4, 4; 4, 4, 4; 4, 4, 4; 4, 4, 4;

With not more than five tentacles in any subdivision: —

4, 5, 5; 4, 5, 4; 5, 5, 4; 4, 4, 4;
4, 3, 3; 3, 3, 4; 4, 3, 3; 3, 5, 5;
4, 3, 5; 3, 3, 3; 3, 3, 3; 4, 3, 5;
3, 5, 4; 3, 4, 3; 4, 3, 3; 3, 3, 3;
4, 4, 4; 4, 4, 5; 4, 3, 3; 4, 4, 4;
3, 3, 3; 4, 3, 3; 3, 5, 3; 3, 4, 3;
3, 3, 3; 4, 3, 4; 4, 4, 4; 4, 5, 4;
3, 3, 3; 4, 4, 5; 4, 3, 5; 4, 3, 5;
4, 4, 3; 5, 3, 4; 4, 5, 4; 5, 4, 3;
3, 5, 4; 4, 5, 4; 5, 5, 4; 5, 4, 3;
3, 4, 3; 5, 4, 5; 4, 4, 3; 3, 3, 5;
3, 4, 5; 5, 3, 4; 5, 4, 3; 4, 5, 4;
3, 4, 4; 5, 4, 4; 5, 4, 3; 5, 5, 5;
3, 3, 3; 5, 2, 4; 2, 3, 4; 4, 3, 2;
3, 5, 5; 4, 5, 4; 4, 5, 5; 4, 4, 4;
4, 4, 4; 5, 4, 4; 4, 4, 5; 4, 5, 4;
4, 4, 5; 5, 4, 5; 5, 5, 5; 5, 5, 4;
3, 3, 4; 5, 5, 5; 4, 5, 5; 5, 4, 3;
*3, 3, 3; 3, 3, 2; 4, 3, 2; 2, , 5, 3;
3, 4, 3; 5, 5, 3; 3, 5, 3; 4, 4, 3;
3, 3, 4; 5, 4, 3; 4, 4, 3; 5, 4, 4;
3, 4, 3; 3, 4, 3; 3, 5, 3; 3, 4, 2;
4, 3, 5; 5, 5, 5; 5, 4, 5; 4, 4, 5;
4, 4, 5; 5, 5, 4; 5, 4, 4; 5, 4, 4;
4, 4, 4; 5, 4, 4; 5, 4, 5; 4, 5, 4;
5, 5, 5; 5, 5, 5; 5, 5, 5; 5, 5, 5;
3, 3, 4; 3, 4, 4; 5, 3, 3; 3, 5, 3;
3, 3, 4; 4, 3, 4; 4, 3, 4; 2, 3, 5;
3, 3, 4; 4, 5, 5; 4, 3, 5; 4, 4, 4;

* All specimens characterized by some peculiar variation are marked *

With not more than six tentacles in any subdivision: —

5, 5, 6; 6, 5, 5; 6, 6, 5; 6, 5, 6;
6, 4, 3; 5, 5, 5; 5, 6, 3; 3, 6, 5;
3, 4, 4; 4, 4, 4; 3, 4, 5; 5, 6, 5;
4, 3, 3; 4, 5, 4; 5, 4, 4; 6, 3, 5;
5, 5, 5; 5, 4, 3; 6, 6, 4; 4, 4, 3;
4, 4, 4; 5, 4, 4; 5, 5, 5; 5, 6, 5;
4, 4, 4; 4, 4, 4; 3, 6, 2; 4, 6, 5;
3, 4, 4; 5, 4, 4; 3, 6, 3; 4, 6, 5;
3, 3, 5; 5, 6, 6; 4, 5, 6; 5, 3, 4;
4, 5, 6; 6, 5, 4; 5, 4, 6; 6, 5, 5;
4, 4, 5; 5, 5, 5; 4, 5, 6; 4, 5, 5;
4, 5, 4; 4, 6, 4; 5, 5, 6; 5, 5, 5;
3, 4, 3; 5, 6, 4; 3, 5, 5; 5, 6, 2;
4, 4, 4; 4, 5, 4; 5, 6, 5; 5, 5, 4;
5, 5, 5; 5, 5, 5; 5, 5, 6; 5, 6, 4;
3, 5, 4; 4, 5, 4; 5, 6, 5; 5, 5, 4;
4, 5, 5; 5, 6, 6; 5, 6, 5; 5, 5, 5;
5, 6, 5; 6, 5, 6; 5, 6, 5; 6, 6, 6;
3, 5, 6; 5, 6, 3; 6, 4, 5; 5, 5, 3;
4, 6, 5; 5, 6, 5; 6, 5, 5; 6, 5, 5;

5, 5, 4; 5, 5, 5; 6, 5, 5; 5, 5, 5;
5, 4, 5; 6, 5, 4; 5, 5, 6; 6, 4, 6;
5, 4, 4; 5, 5, 5; 6, 5, 4; 5, 5, 4;
5, 6, 5; 6, 5, 6; 6, 5, 6; 6, 6, 5;
3, 5, 5; 4, 6, 5; 5, 5, 5; 5, 5, 5;
3, 5, 4; 4, 5, 4; 5, 6, 5; 6, 4, 4;
4, 5, 5; 5, 5, 5; 5, 5, 5; 5, 6, 5;
3, 4, 5; 4, 5, 5; 6, 5, 5; 4, 5, 4;
3, 4, 5; 4, 6, 5; 5, 6, 4; 3, 5, 5;
4, 3, 5; 4, 5, 4; 4, 4, 6; 4, 4, 6;
3, 6, 4; 5, 6, 6; 4, 5, 5; 5, 5, 4;
5, 4, 6; 5, 5, 6; 5, 6, 5; 5, 5, 5;
3, 4, 4; 5, 6, 3; 3, 6, 3; 3, 6, 3;
4, 3, 5; 5, 5, 6; 5, 5, 5; 6, 4, 5;
4, 5, 4; 5, 5, 5; 4, 5, 5; 4, 6, 5;
5, 6, 4; 6, 5, 5; 5, 6, 4; 6, 5, 5;
*3, 3, 3; 3, 3, 4; 5, 3, 4; 3, 6;
5, 6, 5; 5, 6, 5; 5, 6, 5; 5, 6, 5;
6, 5, 6; 6, 5, 6; 6, 5, 6; 6, 5, 6;

Third Stage.

With not more than seven tentacles in any subdivision: —

7, 7, 7; 7, 7, 7; 7, 7, 7; 7, 7, 7; (2)
4, 7, 4; 5, 7, 5; 6, 6, 5; 6, 7, 6;
5, 7, 4; 7, 5, 5; 6, 7, 6; 6, 7, 6;
5, 7, 6; 5, 7, 6; 5, 7, 5; 5, 7, 6;
5, 6, 6; 5, 6, 7; 6, 7, 5; 6, 7, 6;
4, 5, 4; 5, 3, 5; 6, 5, 7; 4, 5, 6;
5, 5, 5; 5, 7, 5; 5, [illegible], 6; 5, 6, 6;
7, 7, 4; 6, 4, 6; 5, [illegible], 7; 6, 6, 7
6, 5, 6; 5, 5, 7; 6, 7, 7; 7, 7, 7;
6, 5, 6; 5, 6, 7; 3, 7, 6; 6, 6, 6;
6, 7, 6; 6, 7, 7; 5, 7, 7; 5, 7, 6;
5, 6, 7; 4, 7, 3; 4, 7, 5; 7, 4, 3;
5, 7, 6; 7, 5, 5; 5, 6, 3; 5, 5, 5;
4, 4, 6; 5, 5, 5; 7, 6, 5; 4, 4, 5;
5, 6, 5; 7, 5, 5; 6, [illegible], 3; [illegible], 5, 5;
3, 5, 5; 5, 4, 5; 7, [illegible], 4; 4, 4, 3;
7, 7, 5; 5, 7, 5; 5, 5, 6; 5, 7, 4;
5, 6, 6; 7, 7, 5; 5, 5, 5; 6, 7, 7;
7, 7, 4; 5, 7, 5; 6, 7, 7; 5, 7, 5;
5, 6, 6; 6, 7, 7; 5, 7, 6; 7, 6, 4;
5, 6, 6; 7, 6, 6; [illegible]; 6, 5, 6;
4, 6, 6; 6, 7, 5; 6, [illegible], 6; 5, 7, 4;
3, 5, 5; 6, 6, 7; 3, 4, 7; 6, 5, 5;

5, 6, 6; 6, 7, 5; 7, 5, 6; 5, 7, 5;
3, 3, 5; 5, 6, 6; 5, 7, 5; 5, 3, 5;
4, 4, 6; 4, 6, 5; 5, 5, 6; 7, 6, 2;
4, 7, 6; 5, 7, 5; 7, 7, 7; 7, 7, 5;
5, 5, 3; 5, 6, 7; 5, 4, 5; 5, 5, 7;
5, 2, 5; 6, 6, 5; 6, 7, 6; 6, 7, 4;
4, 7, 6; 5, 7, 5; 7, 7, 7; [illegible];
5, 5, 4; 5, 6, 6; 5, 5, 5; 4, 7, [illegible];
5, 6, 5; 5, 6, 5; 5, 7, 5; 6, 6, 6;
*5, 5, 4; 6, 7; 7; 5, 7, 6;
5, 6, 6; 6, 6, 5; 5, 7, 6, 5, 7, 7;
4, 3, 7; 5, 6, 6; 6, 5, 5; 6, 7, 4;
5, 6, 5; 6, 7, 6; 5, 7, 5; 6, [illegible], 4;
3, 7, 6; 6, 6, 2; 6, 6, 6; 5, 7, 6;
4, 4, 5; 4, 5, 5; 5, 4, 7; 5, 4, 4;
3, 6, 7; 5, 6, 5; 5, 7, 5; 6, 6, 5;
3, 7, 6; 5, 5, 2; 5, 6, 7; 5, 6, 6;
3, 5, 6; 6, 6, 5; 5, 6, 4; 4, 5, 7;
3, 3, 7; 6, 6, 5; 5, 6, [illegible]; 4, [illegible], 7;
3, 5, 6; 6, 6, 5; 5, 6, [illegible]; 5, 5, 7;
[illegible], 6, 6, 6, 6, 6; 6, [illegible], 5; 6, [illegible], 6;
[illegible], 7, 6; 6, 6, 6; 6, 6, 6, 6, 6, 6;
3, 7, 5; 4, 6, 4; 5, 6, 6; 5, 4, 6;

3, 6, 6; 4, 5, 5; 5, 6, 5; 5, 7, 3;
5, 7, 7; 6, 6, 7; 7, 6, 7; 7, 7, 7;
5, 5, 5; 6, 7, 6; 6, 6, 4; 5, 7, 5;
3, 7, 4; 5, 6, 4; 2, 5, 5; 5, 6, 3;
4, 7, 6; 6, 7, 5; 6, 7, 7; 7, 5, 7;
5, 6, 6; 5, 7, 5; 5, 6, 6; 6, 5, 6;
4, 4, 5; 4, 7, 4; 5, 5, 5; 4, 5, 5;
5, 6, 5; 6, 5, 7; 7, 5, 4; 6, 6, 6;
4, 5, 6; 6, 6, 5; 5, 6, 5; 5, 7, 6;
3, 4, 6; 6, 5, 6; 7, 4, 5; 5, 5, 5;
6, 6, 6; 6, 7, 6; 6, 7, 6; 6, 6, 6;
5, 5, 5; 5, 6, 5; 5, 7, 5; 5, 7, 5;
5, 3, 5; 5, 4, 5; 5, 6, 3; 3, 5, 7;
5, 6, 4; 6, 6, 6; 6, 5, 7; 5, 7, 6;
5, 5, 6; 6, 7, 6; 6, 7, 6; 6, 5, 4;
5, 7, 5; 7, 6, 6; 6, 7, 5; 6, 6, 6;
4, 7, 7; 7, 6, 6; 7, 6, 5; 6, 7, 6;
*5, 6, 6; 6, 6, 6; 6, 6, 6; 3, 3, 2, 3, 7;
4, 7, 7; 7, 7, 6; 6, 7, 7; 7, 7, 5;
5, 7, 6; 6, 6, 6; 7, 7, 6; 7, 7, 6;
5, 6, 6; 6, 7, 7; 7, 7, 6; 7, 7, 6;
5, 5, 7; 6, 5, 7; 7, 6, 6; 6, 7, 6;
5, 7, 5; 5, 7, 6; 6, 7, 6; 7, 7, 5;
*3, 6, 7; 5, 6, 4; 7, 6, 6; , 7, ;
4, 6, 4; 4, 6, 5; 5, 7, 4; 5, 7, 5;
5, 5, 7; 5, 6, 6; 6, 6, 5; 5, 7, 6;
5, 6, 6; 5, 6, 7; 6, 5, 7; 6, 4, 7;
5, 4, 6; 5, 4, 7; 6, 5, 7; 6, 7, 7;
5, 6, 4; 6, 5, 5; 5, 6, 4; 7, 5, 5;
5, 6, 6; 6, 6, 6; 7, 5, 6; 7, 7, 6;
3, 4, 3; 5, 7, 4; 4, 3, 4; 3, 4, 5;
*5, 7, 6; 5, 5, 4; 6, 7; 7;
*6, 5, 6; 7, 7, 5; 5, 3; 2, 3; 5, 5, 6;
*5, 6, 5; 6, 7, 5; 6, 3, 3; 5, 6, 5;
7, 7, 7; 7, 7, 7; 7, 7, 7; 7, 7, 7;
7, 7, 7; 7, 7, 7; 7, 7, 7; 7, 7, 7;
6, 7, 6; 6, 7, 6; 6, 7, 6; 6, 7, 6;

With not more than eight tentacles in any primary subdivision: —

6, 6, 7; 6, 7, 7; 7, 7, 7; 8, 7, 7;
5, 7, 6; 7, 6, 5; 6, 6, 5; 8, 7, 6;
6, 7, 5; 7, 7, 7; 8, 5, 4; 6, 7, 6;
3, 5, 4; 5, 5, 8; 5, 7, 8; 6, 7, 5;
4, 7, 6; 5, 7, 5; 8, 7, 7; 7, 7, 5;
*3, 5, 3; 6, 3, 4; 4, 5, 5; 8, 4;
7, 8, 7; 7, 8, 8; 8, 7, 8; 8, 7, 8;
*3, 3, 3; 3, 3, 4; 3, 5, 4; 8, 3;
5, 6, 7; 6, 7, 6; 8, 5, 5; 7, 5, 5;
5, 5, 5; 5, 7, 7; 6, 7, 5; 8, 5, 5;
4, 5, 5; 8, 4, 5; 5, 5, 5; 5, 5, 6;
*3, 3, 2; 4, 3, 1, 3; 4, 8, 2; 2, 3, 1;
3, 7, 5; 6, 7, 8; 5, 6, 6; 7, 7, 4;
5, 7, 7; 6, 8, 6; 6, 6, 6; 7, 7, 7;
5, 7, 7; 7, 7, 7; 6, 7, 7; 6, 7, 8;
5, 5, 6; 4, 7, 8; 7, 6, 5; 5, 6, 7;
5, 5, 7; 7, 6, 6; 6, 7, 6; 8, 5, 6;
5, 5, 5; 6, 6, 6; 5, 6, 6; 7, 8, 3;
5, 5, 5; 5, 6, 5; 5, 6, 5; 8, 7, 6;
5, 5, 6; 5, 8, 3; 4, 7, 6; 5, 7, 5;
6, 6, 7; 6, 6, 7; 8, 7, 6; 6, 7, 7;
*2, 3, 3; 3, 1, 3, 4; 1, 3, 2; 2, 8, 4;

With not more than nine tentacles in any subdivision: —

7, 3, 7; 9, 7, 5; 5, 7, 6; 6, 6, 5;
*6, 6, 7; 7, 7, 7, 9, 7; 7, 7, 7,
3, 9, 5; 7, 5, 5; 6, 5, 5; 5, 7, 7;
5, 3, 5; 5, 6, 5; 6, 3, 9; 5, 6, 6;
4, 7, 7; 5, 8, 7; 6, 9, 4; 7, 8, 7;
*3, 7, 5; 4, 7, 4; 4, 7, 4; 9, 4;
*4, 4, 3; 5, 6; 9, 4; 4, 4, 5;
6, 8, 6; 7, 7, 8; 8, 7, 7; 7, 9, 8;
*7, 4, 6; *7, 6, 4; 7, 9; 7, 5, 7; 7, 6, 6;
*3, 5, 3; 4, 5, 3; 1, 9, 5; 8, 3, 4;

With not more than ten tentacles: —

*2, 6, 6, 6, 9; 5, 6, 5; 7, 6, 10;
6, 8, 5; 7, 8, 8; 7, 4, 6; 10, 8, 9;
5, 10, 6; 7, 8, 6; 6, 6, 8; 6, 7, 8;
*5, 4, 5; 6, 5, 5; 6, 7, 5; 10, 5;
*3, 4, 4; 10, 4; 3, 5, 5; 4, 6, 6;
*5, 6, 7; 9, 5, 2; 3, 10; 8, 7, 6;

With not more than eleven tentacles: —

*11, 5; 11, 5; 5, 5, 5; 6, 5, 4;
2, 11, 5; 5, 9, 4; 5, 10, 3; 5, 7, 3;
8, 7, 9; 9, 7, 8; 11, 10, 10; 11, 7, 10;
*3; 11, 6; 5, 4, 2; 8, 8, 4; 7, 7, 8;
3, 11, 10; 6, 4, 8; 9, 8, 10; 6, 8, 4;
*4, 6, 5; 2, 8, 6, 7; 1, 11, 2;
*3, 5, 5; 4, 5, 6; 4, 11; 11, 2;
7, 8, 11; 10, 7, 7; 11, 6, 7; 11, 7, 6;

With not more than twelve tentacles: —

*5, 5, 5; 5, 6, 6; 7, 6, 6; 12, 7;
*6, 7, 6; 6, 7, 7; 7, 7, 7; 12, 5;
*5, 7, 4; 4, 7, 6; 5, 7, 4; 12, 5;
*3, 6, 2; 3, 3; 10, 9, 9; 10, 7, 8;
8, 7, 12; (2 forks).

With not more than thirteen tentacles: —

*5, 6, 4; 6, 13; 6, 7, 4; 5, 7, 7;
*8, 3, 13; 5; 11, 8, 11; 12, 7, 10;
*4, 4, 5; 6, 4, 4; 13, 5; 5, 6, 4;
*9, 7, 8; 10, 7, 10; 13, 9, 7;

With not more than fourteen tentacles: —

*7, 7, 7; 8, 7, 8; 5, 7, 6; 14, 7;
*7, 6, 5; 7, 8; 1,, 10; 14;
*5, 7, 7; 6, 7, 6; 7, 8, 7; 9, 14;

Fourth Stage.

With not more than fifteen tentacles: —

*2, 5, 5; 2, 15, 6, 7; 9, 10; 9, 2;
*6, 6, 7; 6, 15; 6, 2; 1, 8, 6;
*5, 6, 5; 15, 6; 6, 7, 7; 7, 6, 7;
*6, 7, 10; 6, 7, 15; 12, 14, 5;
*7, 8, 6; 6, 15; 3, 15; 7, 8, 7;

With not more than seventeen tentacles: —

*17; 15; 15; 16; no otoliths.

With not more than eighteen tentacles: —

*6, 6, 6; 7, 7; 9; 18, 7;
*9, 7, 9; 18, 7; 11, 17; 11, 18;

With not more than twenty tentacles: —

*7, 6, 7; 7, 7, 8; 8; 1, 8; 20, 8;

With not more than twenty-seven tentacles: —

*9, 6, 8; 27; 8, 11;

Table showing Variations in the number of Radial Canals, of Quadrants, of Primary Divisions of Quadrants, or of the Otoliths.

Irregular.	Number of Otoliths.	Number of Radial Canals.
6, 7; 7; 5, 7, 6; 5, 5, 4;	5	4
11, 5; 11, 5; 5, 5, 5; 6, 5, 4; . . .	6	4
5, 7, 6; 14, 7; 7, 7, 7; 8, 7, 8; . . .	7	4
5, 5, 5; 5, 6, 6; 7, 6, 6; 12, 7; . . .	7	4
3, 5, 3; 6, 3, 4; 4, 5, 5; 8, 4; . . .	7	4
6, 7, 6; 6, 7, 7; 7, 7, 7; 12, 5; . . .	7	4
1, 3, 3; 3, 3, 3; 3, 3, 3; 3, 3; . . .	7	4
6, 6, 7; 7, 7, 7; 9, 7; 7, 7, 7; . . .	7	4
5, 7, 4; 4, 7, 6; 5, 7, 4; 12, 5; . . .	7	4
5, 6, 4; 6, 13; 6, 7, 4; 5, 7, 7; . . .	7	4
6, 6, 6; 7, 7; 9; 18, 7;	4	4
8, 3, 13; 5; 11, 8, 11; 12, 7, 10; . .	6	4
2, 5, 5; 2, 15, 6, 7; 9, 10; 9, 2; . .	7	4
3, 6, 2; 3, 3; 10, 9, 9; 10, 7, 8; 8, 7, 12;	9	5
2, 6, 6; 6, 9; 5, 6, 5; 7, 6, 10; . . .	7	4
3, 3, 3; 3, 3, 4; 3, 5, 4; 8, 3; . . .	7	4
7, 6, 5; 7, 8; 1, , 10; 14;	5	4
3, 3, 3; , 3, 3, 2; 4, 3, 2, 2; , 5, 3; .	9	4
3; 11, 6; 5, 4, 2; 8, 8, 4; 7, 7, 8; . .	7	5
3, 3, 2; 4, 3, 1, 3; 4, 8, 2; 2, 3, 1; .	9	4
5, 6, 6; 6, 6, 6; 6, 6, 6; 3, 3, 2, 3, 7; .	10	4
6, 6, 7; 6, 15; 6, 2; 1, 8, 6;	6	4
3, 7, 5; 4, 7, 4; 4, 7, 4; 9, 4; . . .	7	4
3, 6, 7; 5, 6, 4; 7, 6, 6; , 7 ,; . . .	8	4
17; 15; 15; 16;	0	4
5, 4, 5; 6, 5, 5; 6, 7, 5; 10, 5; . . .	7	4
4, 6, 5; 2, 8, 6, 7; 1, 11, 2;	7	3
4, 4, 5; 6, 4, 4; 13, 5; 5, 6, 4; . . .	7	4
4, 4, 3; 5, 6; 9, 4; 4, 4, 5;	6	4
3, 4, 4; 10, 1; 3, 5, 5; 4, 6, 6; . . .	7	4
3, 5, 5; 4, 5, 6; 4, 11; 11, 2; . . .	6	4
9, 7, 9; 18, 7; 11, 17; 11, 18; . . .	5	4
5, 6, 5; 15, 6; 6, 7, 7; 7, 6, 7; . . .	7	4
5, 6, 7; 9, 5, 2; 3, 10; 8, 7, 6; . . .	7	4
7, 4, 6; 7, 6, 1; 7, 9; 7, 5, 7; 7, 6, 6;	9	5
6, 7, 10; 6, 7, 15; 12, 14, 5; . . .	6	3
9, 7, 8; 10, 7, 10; 13, 9, 7;	6	3
5, 7, 7; 6, 7, 6; 7, 8, 7; 5, 7, 7; 9, 14;	9	4
3, 3, 3, 3, 3, 4; 5, 3, 4; 3, 6; . . .	7	4
7, 8, 6; 7, 8, 7: 6, 5; 3, 15;	6	4
5, 7, 6; 5, 5, 4; 6, 7; 7, ;	6	4

Irregular.	Number of Otoliths.	Number of Radial Canals.
7, 6, 7; 7, 7, 8; 8; 1, 8; 20, 8; . .	6	5, 1 forking.
9, 6, 8; 27; 8, 11;	3	3
6, 5, 6; 7, 7, 5; 5, 3; 2, 3; 5, 5, 6; .	8	5
7, 6, 5; 7, 8; 1, , 10; 14; . . .	5	4
2, 3, 3; 3, 1, 3, 4; 1, 3, 2; 2, 8, 4; .	9	4
6, 6, 7; 6, 15; 6, 2; 1, 8, 6;	6	4

On Plate IX. will be found a number of diagrammatic figures giving an idea of the irregular growth of the marginal tentacles. The lengths of the tentacles are drawn as fully expanded, the position of the radial and circular canals is indicated, and the otolith tentacles are marked by a cross. The structure and length of the marginal tentacles of Eucope are such that the comparative length of adjoining tentacles is readily observed, owing to the slight degree of contraction and expansion they possess.

In Figures 1 to 6, 10, and 14 (Plate IX.), we have the normal number of tentacles (seven) in each of the primary quadrantic subdivisions. It is noticeable that t_1 can only in the case of Figures 4 and 10 be distinguished from the two t_2, while the four t_3 are of nearly uniform size in all the figures except Figure 6.

In Figures 7, 12, 13, 16, 17 (one primary division), 18, 19, and 22–26, there are only five marginal tentacles in each primary quadrantic division. In the greater number of these figures it is possible to distinguish t_1, or t_1 and t_2, while the irregularly developed tentacles are part of the t_3 cycle. In Figures 20–22 (one sector), and 27–29 (two sectors), the cycles t_1 and t_2 can be distinguished, and the irregularity of development occurs in the t_3 cycle, which may appear at different points of the circular canal, as is seen by comparing Figures 20–22, 27, and 28.

Figures 8–10, 17, 22, 29, and 30 show the irregularity in time of the development of the marginal tentacles in the different sectors of the same quadrant, as well as the irregularity in the growth of the three cycles t_1–t_3 in adjoining sectors. In Figures 8, 29, and 30, the marginal tentacles between the otoliths are in the same stage of growth, but the tentacles of the right or left sectors are in very different stages of growth. In one case (Fig. 30) only one t_1 is developed in each sector; in Figure 29 the cycles of t_1 and t_2 are normal, while they are most irregular in the sectors of Figure 8.

When six marginal tentacles occur in one sector the irregularities in the t_2 and t_3 cycles are very marked (see Figs. 8, 9, 11, 15, and 17); t_1 can only be distinguished from the t_2 pair by its position.

The coalescence at the base of adjoining marginal tentacles to form a double tentacle with two spurs and two lashes is not uncommon. During the summer there were fourteen specimens met with having double tentacles; in all except two cases they were connected with the tentacle riding a sense organ.

The sensory tentacles usually have only one otolith; we however observed thirteen cases in which each sense organ contained two (Plate VIII. Figs. 15, 17), and five in which there were three otoliths (Plate VIII. Figs. 14, 16), and one in which there were no otoliths in any of the quadrants.

An examination of the table on page 137, in which the more interesting of the variations observed have been collected, will show how large a number of specimens show great variation in the number of the sense-bearing tentacles. Among specimens of Eucope with the normal number of quadrants we find the otoliths bearing tentacles vary from eight, the normal number, two in each quadrant, to three on one side and ten on the other. As will be noted, there are only five cases in which the sensory tentacles are greater in number than in the norm, while the number of cases in which they are suppressed is quite large. Their increase does not always accompany an increase in the number of radial canals. Two out of four specimens with five radial canals possessed nine sensory tentacles, another only seven, and one eight.

There seems to be no correlation between the number of marginal tentacles in any sector and the number of sensory tentacles.

The primary sector with the largest number of tentacles has often only one sensory tentacle, while that with a smaller number has two.

In a specimen with quadrants of unequal size, and with an unequal number of marginal tentacles, in each of which the formula is

7; 6; 4, 5, 5; 6, 7, 5;

there are six otoliths, one on each of the tentacles at the base of the radial canals between the first and second and the second and third quadrants, and the others as marked by the comma in the third and fourth quadrants.

In another specimen with very unequal quadrants there are nine otoliths, there being three in the largest quadrant, as shown by its formula. the first quadrant being the smallest, the last the largest:

1, 3, 2; 2, 8, 4; 2, 3, 3; 3, 1, 3, 4;

In a specimen in which two small quadrants are adjacent, there are only six otoliths. Taking the two smaller quadrants first, the formula is

6, 2; 1, 8, 6; 6, 6, 7; 6, 15;

The last quadrant is the largest.

The formula of another specimen, with unequal quadrants, is,

6, 6, 6; 7, 7; 9; 18, 7;

This shows only four otoliths, one quadrant without any, and two with only one.

In a similar specimen with a formula of

11, 8, 11; 12, 7, 10; 8, 3, 13; 5;

there are only six otoliths, with three normal quadrants, one not having any sense organs.

In a specimen with two adjoining radial canals forking below the genitals, making four quadrants with two small sectors cut out of two of them, there are ten otoliths, the formula being

$$10, 9, 9;\ 10, 7, 8;\ 2, 6, \overbrace{2,\ 8,}\ 7, 12;\ 3, \overbrace{3;\ 10,}\ 1;$$

In a second specimen, forking similarly at the extremity of one radial canal, the formula was

$$7, 6, 7;\ 7, 7, 8;\ \ 8;\ \overbrace{1, 8;\ 20, 8;}$$

In a specimen with an eccentric digestive cavity and quadrants unequally developed, the formula is

14; 7, 6, 5; 7, 8; 1, , 10;

or only five otoliths, neither the first nor the third quadrant having otoliths, while the fourth has two side by side.

In a specimen with equally developed quadrants, but with a long spur above the genitals at right angles to the radial canal, barely reaching the marginal canal, the formula is

$$\overbrace{3;\ 11, 6;}\ 5, 4, 2;\ 8, 8, 4;\ 7, 7, 8;$$

there being three tentacles between the fork (spur) and the nearest canal.

In a very irregularly developed specimen, with the formula

6, 6, 6; 7, 7; 9; 18, 7;

there were only three otoliths.

In a specimen with only three radial canals, one of which forks above the genitals, the formula is

$$2, 15, 6, 7 ;\ 3, 10, 1 ;\ 9, \overbrace{2 ;\ 2}, 5, 5 ;$$

the otoliths being normal in number, but irregular in their distribution.

In another Eucope with three radial canals, we find seven otoliths represented in the formula

1, 11, 2 ; 4, 6, 5 ; 2, 8, 6, 7 ;

the last sector being somewhat larger than the others.

In a specimen with three equally developed sectors with the formula of

12, 14, 6 ; 6, 7, 10 ; 6, 7, 15 ;

there are six otoliths, the normal number in each sector, but the other marginal tentacles are most irregularly developed.

In still another Eucope, similar to the preceding one, with three sectors of equal size and well developed genitals with six otoliths, two in each sector, the formula was

10, 7, 10 ; 10, 9, 7 ; 9, 7, 8 ;

Finally, a Eucope with three quadrants of the same size had only three otoliths ; its formula is

9, 6, 9 ; 27 ; 8, 9 ;

The formation of spurs (Plate VIII. Figs. 4–13) takes place usually at the base of the marginal tentacle, at its connection with the circular canal, but cases have been observed in which the spur shoots off from the lash of the tentacle (Plate VIII. Fig. 5). The formation of spurs is often accompanied by the atrophy of the inframarginal knob of the marginal tentacles (compare Plate VIII. Figs. 9–13, with Figs. 4–8).

It will be noticed that in the numerical variation of the segments the tendency is not to doubling the number of normal (4) segments, but either to add one or two, or to reduce the segments to three.

In Sarsia, Agassiz observed a specimen with six radial canals and Romanes one with five ; he also observed a Sarsia with six radial canals, six ocelli, and six tentacles, like that seen by Agassiz, the only specimen in thousands examined.

According to Bateson, the numerical variations in Aurelia tend in two directions, i. e. to forms with six and twelve segments instead of the normal eight.

The same tendency in Aurelia to vary in the direction of six and twelve segments was pointed out by Brown, and was also noted in the older papers of Ehrenberg and Romanes.

According to Romanes, monstrous forms of Aurelia aurita are of frequent occurrence. Abnormality consisted in multiplicity and abortion of parts. All cases of asymmetrical multiplication applied to lithocysts, and always occurred in the same manner. When there were nine instead of eight lithocysts, the extra one was always fully developed and in close proximity to one of the normal lithocysts.

In symmetrical abnormalities all parts of the organism were equally affected. Thus all examples of multiplication extended proportionally to ovaries, nutritive canals, lithocysts, and tentacles, the effect being to increase the number while adhering to the type of the natural segments. In all cases the degree of abnormality was the same; e. g. 6 ovaries, 24 unbranched radial tubes, 12 lithocysts, and a six-lobed manubrium. All parts and segments thus increased one third their normal number. Romanes calls attention to the fact that this is the same proportional increase as in Sarsia, with six canals, and explains it as accidental. Supernumerary lithocysts always occur at the ends of the faintly colored radial tubes, never at the ends of the darker ones.

Segments and lobes of the manubrium may be multiplied without the ovaries increasing in number. Again, segments may multiply and manubrium and ovaries remain normal. Processes of multiplication may not extend to all quadrants of the umbrella. Multiplication of parts may be confined to one side of the umbrella, thus doubling or tripling organs on one side only.

Abnormalities usually are symmetrical. When they are not, the manubrium and ovaries are not affected, the segments only being multiplied. Abortion of parts takes place in the same symmetrical way as multiplication: there may be one ovary and six segments, and three ovaries instead of eight and four. Segments and ovaries may also be reduced to one half the normal number. In these two cases the manubrium is not affected. Abortion of parts was observed in the ovaries only. Partial suppression of ovaries was of frequent occurrence. The most prevalent case was where one ovary was smaller than the other three. Reduction also occurs in two alternating ovaries (i. e. opposite?). Sometimes three adjacent ovaries were reduced in size.

Total suppression of one ovary was more rare. Only in twelve cases in thousands was total suppression of two ovaries observed: sometimes it was two adjacent ones, and more frequently the two opposite ones that were absent. In one case three ovaries were absent, the specimen being otherwise fully developed. In no case was it observed that deficiency or absence of ovaries entailed a corresponding deficiency or absence of other organs. Reduction or suppression does not occur in any other organ than ovaries in A. aurita.

H. C. Sorby found, among A. aurita collected in Suffolk and Essex, a "few per thousand" abnormal specimens exhibiting sixfold, fivefold, threefold, and partial twofold symmetry. References to variations in Aurelia, Clavatella, Sarsia, and Stomobrachium may be found also in Bateson's "Materials for the Study of Variation," pp. 421–429. Edward T. Browne examined 383 specimens of A. aurita. He found that eight specimens (2.08%) exhibited numerical variations in the genital sacs, buccal arms, and tentaculocysts. The number of the genital sacs and of the buccal arms varied from three to six. He concludes that there appeared to be a correlation between genital sacs and buccal arms, but that the tentaculocysts vary independently of these. Eighty-seven cases (22.8%) showed variation in the number of tentaculocysts. Twenty of these had less, and the remainder more, than the normal number. The range of variation in tentaculocysts was 6 to 15.

The preceding observations on the variations of Aurelia show some striking differences from those we have made on Eucope. While in Aurelia there is a general correlation between the number of segments of genital sacs, of buccal lobes, and of tentaculocysts, there is no such correlation in the variations of Eucope. The sense organs in Eucope vary, both in number and in position, irrespectively of the number of radial canals and of segments. Neither multiplication nor abortion of parts in Eucope is symmetrical. The suppression of genital sacs is quite common in Eucope, while it is rare in Aurelia. In Eucope suppression is not limited to genital sacs; as in Aurelia, it extends to the otolith-bearing tentacles. As far as we have observed, the number of terminal folds of the manubrium does not vary in Eucope, and is not correlated to the number of segments.

The apparatus used in making the photographs was the large photomicrographic apparatus of Zeiss, with some modifications, direct sunlight being employed by means of a heliostat of the automatic kind, and all exposures were instantaneous. The camera was always used in the horizontal position, so that with an objective of low power the full length of the bellows could be employed to obtain sufficient magnification with the least loss of light. The objectives employed in photographing Eucope were those of 35 and 70 mm. focus, the lower power being employed with the larger specimens. The exposures were made with a "Low" pneumatic shutter, this model being chosen on account of the small amount of space it occupies, allowing it to be introduced between the camera and the microscope. This was accomplished by clamping the shutter to the collar on the front-board of the camera, another similar collar being screwed to the front of the shutter for the light tight connection with the microscope. With the microscope in the horizontal position the light was taken directly from the mirror of the heliostat and diffused by means of a disk of blue ground-glass placed in the substage immediately behind the iris diaphragm, and then passes through a simple condensing lens to the object on the stage of the microscope. The immediate source of the light, the ground-glass, is thus brought near to the object to be photographed, giving a brilliant illumination and permitting the use of a small diaphragm.

The most difficult task was to confine the animals to be photographed, more particularly with the microscope in the horizontal position. The device which proved most serviceable for flat or discoidal objects was a parallel compressor of the model of Hermann Fol. Rings were cut from pure rubber tissue of different thicknesses, the ring to be employed for any particular object being a little thicker than the object itself. The rubber ring was then placed on the lower plate of the compressor and pressed into contact with it by means of the finger. The object is then brought into the centre of the ring, and water added with a pipette until the inside of the ring is completely filled, and the upper part of the compresser carefully screwed down until it comes in contact with the rubber, the superfluous water being at the same time squeezed out. If this be done with care, the inside of the ring will be completely filled with water and contain no air bubbles. There should be just enough pressure to allow the upper glass of the compressor to come in contact with the object. This can be determined by holding the compressor vertically, and screwing down the upper plate until the object ceases to sink. The compressor can now be clamped to the stage of the microscope in any position. By employing rubber rings of sufficient thickness, aquaria can be contrived in this way one eighth of an inch in depth. In photographing the rounder and plumper forms, any pressure upon the animal would produce a sensible change in shape. Such forms, therefore, were placed in small deep watch glasses and confined by glass rings, the microscope being placed in a vertical position, the camera however remaining horizontal. The connection between the microscope and camera was effected by means of the prism end of an Oberhäuser's camera lucida, to which the light tight collar had been adjusted by means of an adapter. The light in this case was centred upon the substage mirror of the microscope, and thus upward. Jellyfish that have been for some time in a small quantity of sea water become partially stupefied by the consumption of the air in the water, and are then more quiet and their tentacles are better extended.

For larger objects, such as Medusæ and Ctenophora, a Zeiss series II.^{a} 1 : 8 photographic lens with an iris shutter was made use of. A reversing prism fastened to the front of the lens allows the use of a horizontal camera in photographing animals in open dishes. Work of this description is done out of doors, illumination being obtained by a series of mirrors, the arrangement of which varies with the nature of the object and the view desired. The work is still in an experimental stage, and it is hoped to give in a subsequent paper a more detailed account of methods and results.

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EXPLANATION OF PLATES.

Plates I.-VI., from photographs taken by W. McM. Woodworth

PLATE I.

To illustrate the variation in the development of the genital organs. The diameter of the disk of the specimens of Eucope figured varied from 3.5 to 4.5 mm.

Fig. 1. Adult male, with equally developed genitals, their formula being 1, 1, 1, 1,

Fig. 2. Adult male, with only three fully developed genitals, their formula being 1, 1, 1, 4,

Fig. 3. Male, with the genital formula 1, 2, 3, 4, all in different stages of development.

Fig. 4. Female with two atrophied genitals, formula being 1, 1, 0, 0,

Fig. 5. Male of the formula 1, 1, 3, 4,

Fig. 6. Male with only one large genital pouch, with the formula 1, 4, 4, 4,

PLATE II.

Fig. 1. Male, with two atrophied genital organs, and with unequally developed radial canals, three of which have spurs running at a slight angle from them.

Fig. 2. Male, with unequally developed genitals, and with one radial canal forking both above and below the genital pouch.

Fig. 3. Young Eucope with undeveloped genital organs.

Fig. 4. Male Eucope with spurs on two of the radial canals, with unequally developed genitals.

Fig. 5. Female with only two fully developed genital pouches, and one atrophied radial canal.

Fig. 6. Male with genital formula 1, 1, 1, 0,

PLATE III.

Figures of Eucope having three or more radial canals, and forking either above or below the genital organs.

Fig. 1. Eucope with three radial canals, one of which forks at the extremities of the heart-shaped genital pouch.

Fig. 2. Male Eucope with four radial canals, also forking at the genital pouch. Nearly equally developed genitals.

Fig. 3. Female Eucope with three radial canals, one of which sends off a prominent spur above the rudimentary genital pouch. Genitals very unequally developed.

Fig. 4. Male with three radial canals, one of which forks well above the genital organs of the branches. Genital organs nearly equally developed.

Fig. 5. Male with four radial canals. The genital organs corresponding to the forks of one of the canals are barely united. Genital pouch atrophied in one quadrant.

Fig. 6. Male Eucope with three unequally developed genital organs.

PLATE IV.

With figures of Eucope having five or more radial canals.

Fig. 1. Female Eucope with five radial canals and the same number of unequally developed genital pouches, with the formula 1, 2, 3, 4, 5,

Fig. 2. Young male Eucope with five radial canals, unequally developed genitals, and a sixth rudimentary radial canal.

Fig. 3. Female Eucope with five radial canals, two fully developed genital pouches, one less so, and two radial canals without genital organs.

Fig. 4. Female Eucope with five radial canals, and only one fully developed genital pouch.

Fig. 5. Female Eucope with five radial canals, one of which is atrophied below the genital pouch. Genital organs unequally developed.

Fig. 6. Female Eucope with five radial canals and unequally developed genitals, one of the canals having no genitals, and forking.

PLATE V.

Fig. 1. Male Eucope with five radial canals and five nearly equally developed genital pouches

Fig. 2. Male Eucope with five radial canals and traces of a sixth, and very unequally developed genitals.

Fig. 3. Female Eucope with five radial canals subdividing the disk into very unequal segments, only two of the genital organs fully developed.

Fig. 4. Female Eucope with unequally developed genitals, but with five very symmetrical segments.

Fig. 5. Female with five radial canals, one of which forks at the periphery, and five very unequally developed genitals.

Fig. 6. Male with five radial canals, unequally developed genitals, and segments of unequal size.

PLATE VI.

Fig. 1. Male Eucope with five radial canals, one of which forks above the genitals close to the base of the digestive cavity, dividing the disk into six different-sized sectors.

Fig. 2. A male Eucope similar to Figure 1, with more fully developed genitals.

Fig. 3. Young Mnemiopsis Leidyi A. Ag., magnified $\frac{1}{3}$.

Fig. 4. Doliolum sp. in profile, magnified $\frac{30}{1}$.

Fig. 5. Annellid larva (Aricidea ?), magnified $\frac{100}{1}$.

Fig. 6. Ectopleura ochracea A. Ag., seen in profile, magnified $\frac{80}{1}$.

PLATE VII.

To illustrate the formation of spurs, branches, and anastomosing canals from the sides of the radial canals.

Fig. 1. Slightly projecting spurs on side of radial canal.

Figs. 2, 3, 6, 7, and 10 show quite prominent hook-like lateral prolongations from the sides of the radial canals.

Figs. 4, 5, and 9 show forks of the radial canals in Figure 4 above the genitals, in Figure 5 below.

Fig. 4 shows a transverse canal connecting adjoining radial canals.

Fig. 8 shows a circular canal connecting the radial canals below the base of the manubrium.

PLATE VIII.

Fig. 1. Marginal sense-bearing tentacle with three otoliths.

Figs. 2 and 3 show the coalescence of adjoining marginal tentacles, Figure 2 with one otolith, Figure 3 with two.

Figs. 4–13 show the mode of formation of an abnormal basal spur frequently seen jutting out from the marginal tentacles, either with or without otoliths.

Figs. 14–17 show the numerical variation of the otoliths in the sense organ of the marginal tentacle.

Fig. 18 shows the coalescence of male genital organs of adjoining radial canals.

Fig. 19 shows the formation of a radial canal shooting up from the circular canal.

Fig. 20. An abnormal Eucope with confluent radial canals and ovaries developed on the main and the lateral canal.

Fig. 21 shows lateral leaf-like expansions of male genital organs which have not as yet run together, as in Figure 18.

PLATE IX.

To illustrate the irregular development of the marginal tentacles belonging to the t_1, t_2, and t_3 cycles.

Figs. 1–6, 10, and 14 are normal sectors belonging to the third stage of development.

Figs. 7–9, 12, 13, 16–19, and 22–26 show sectors belonging to the same stage of development, but there has been great irregularity in the succession and growth of the third cycle of tentacles.

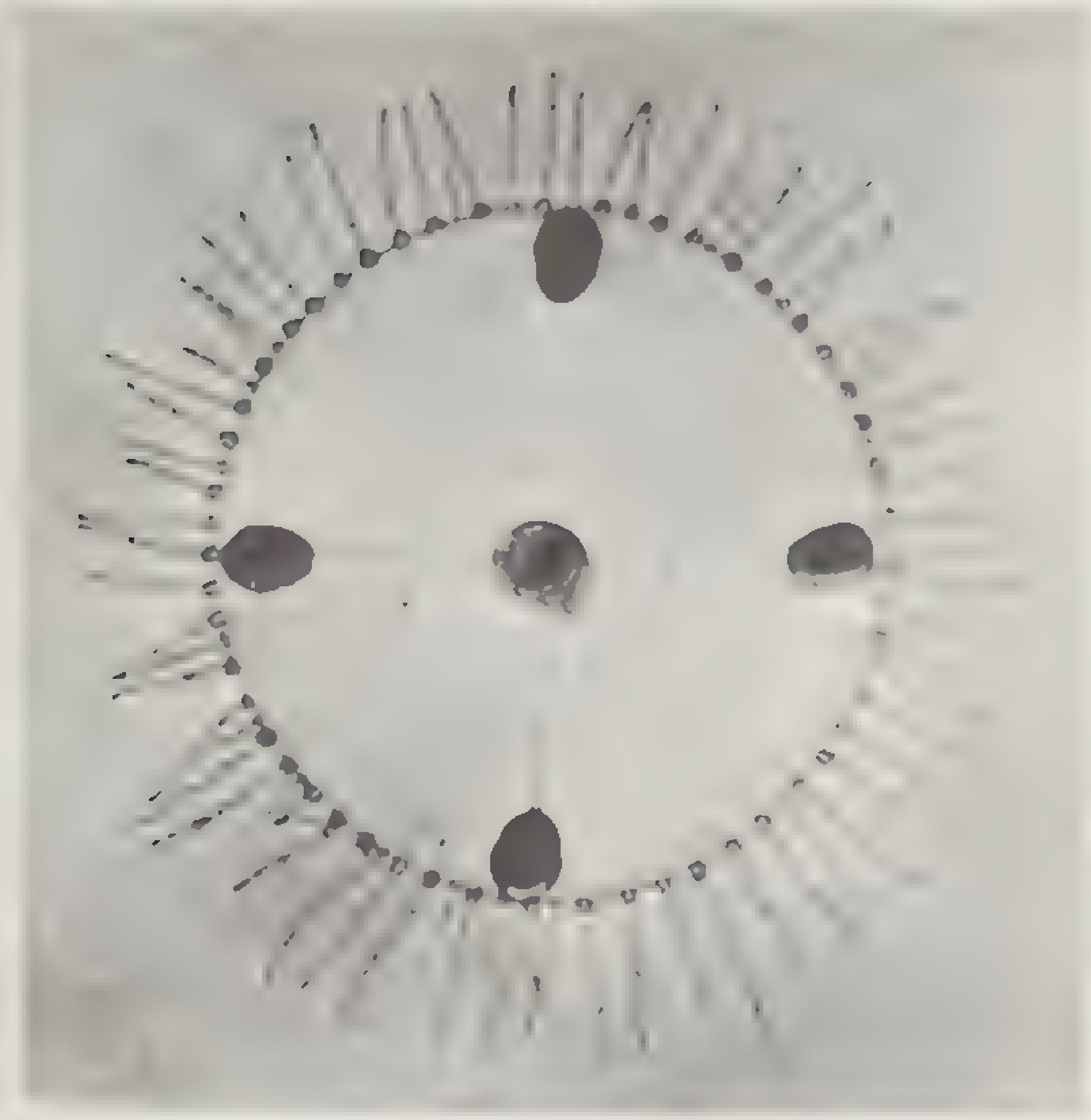
Figs. 20–22, 27, and 28 show sectors with only one tentacle of the t_3 cycle in each primary division.

Figs. 29 and 30 belong to the second stage of development.

EUCOPE

PLATE I.

1



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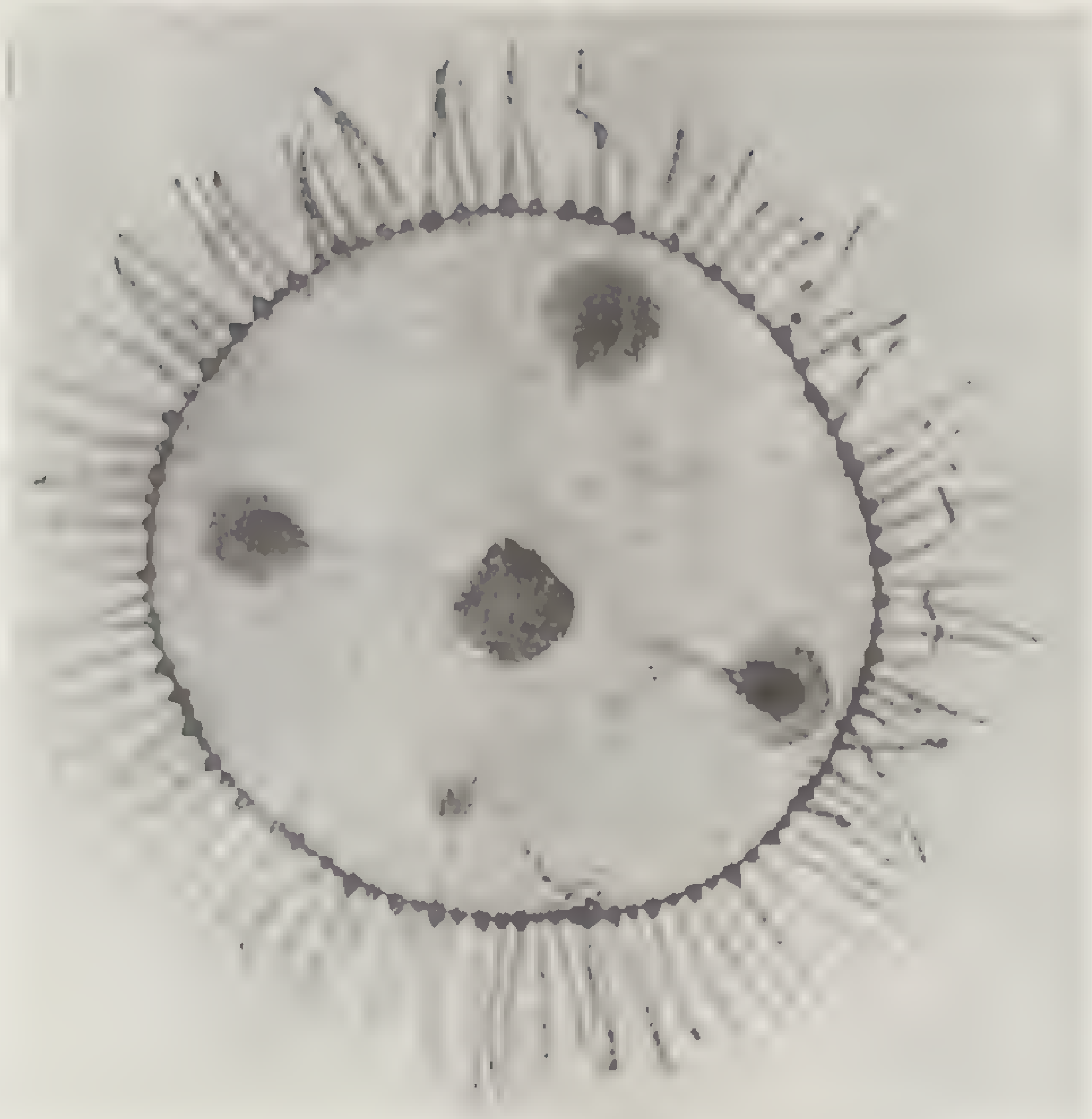

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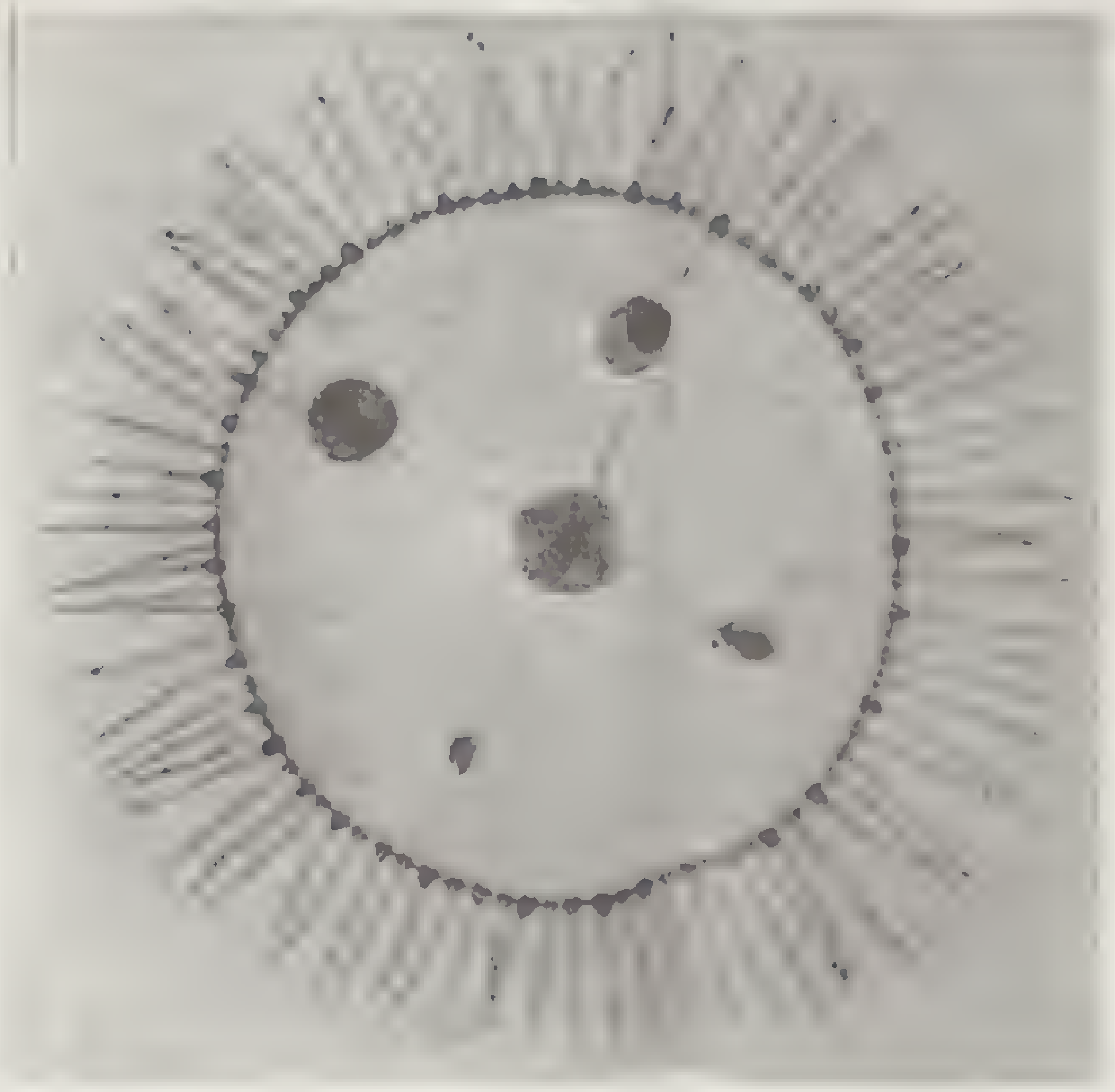

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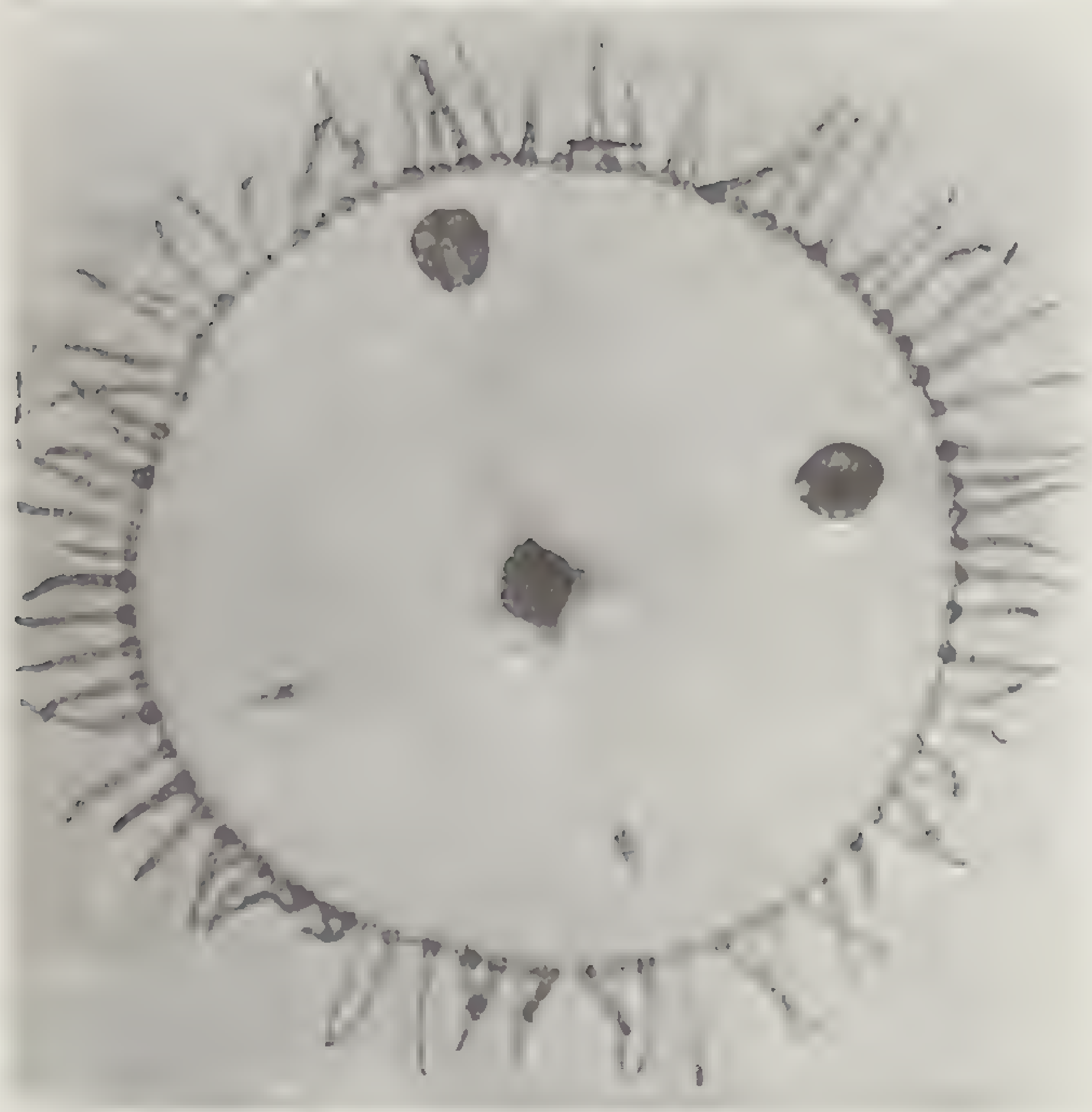

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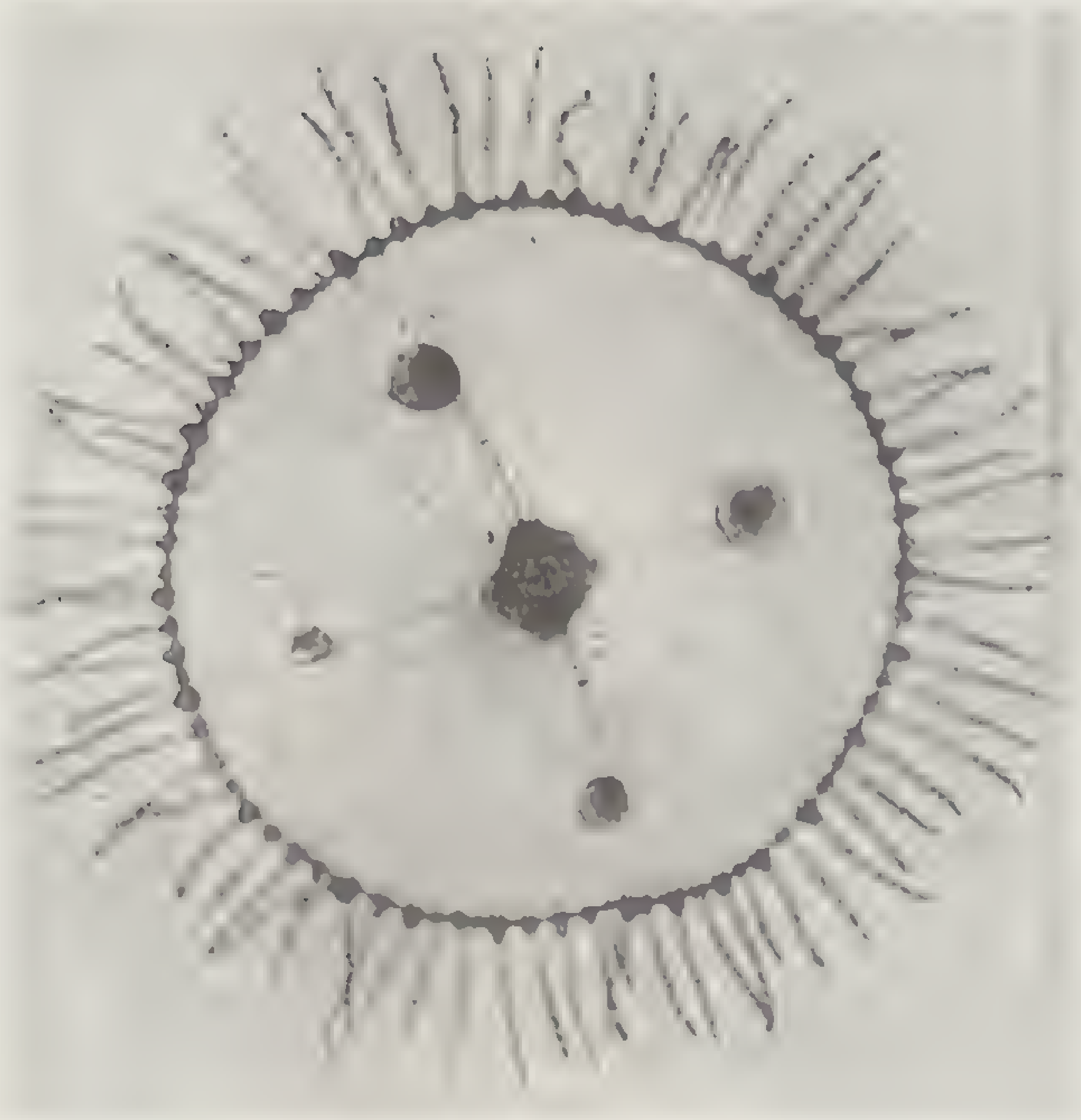

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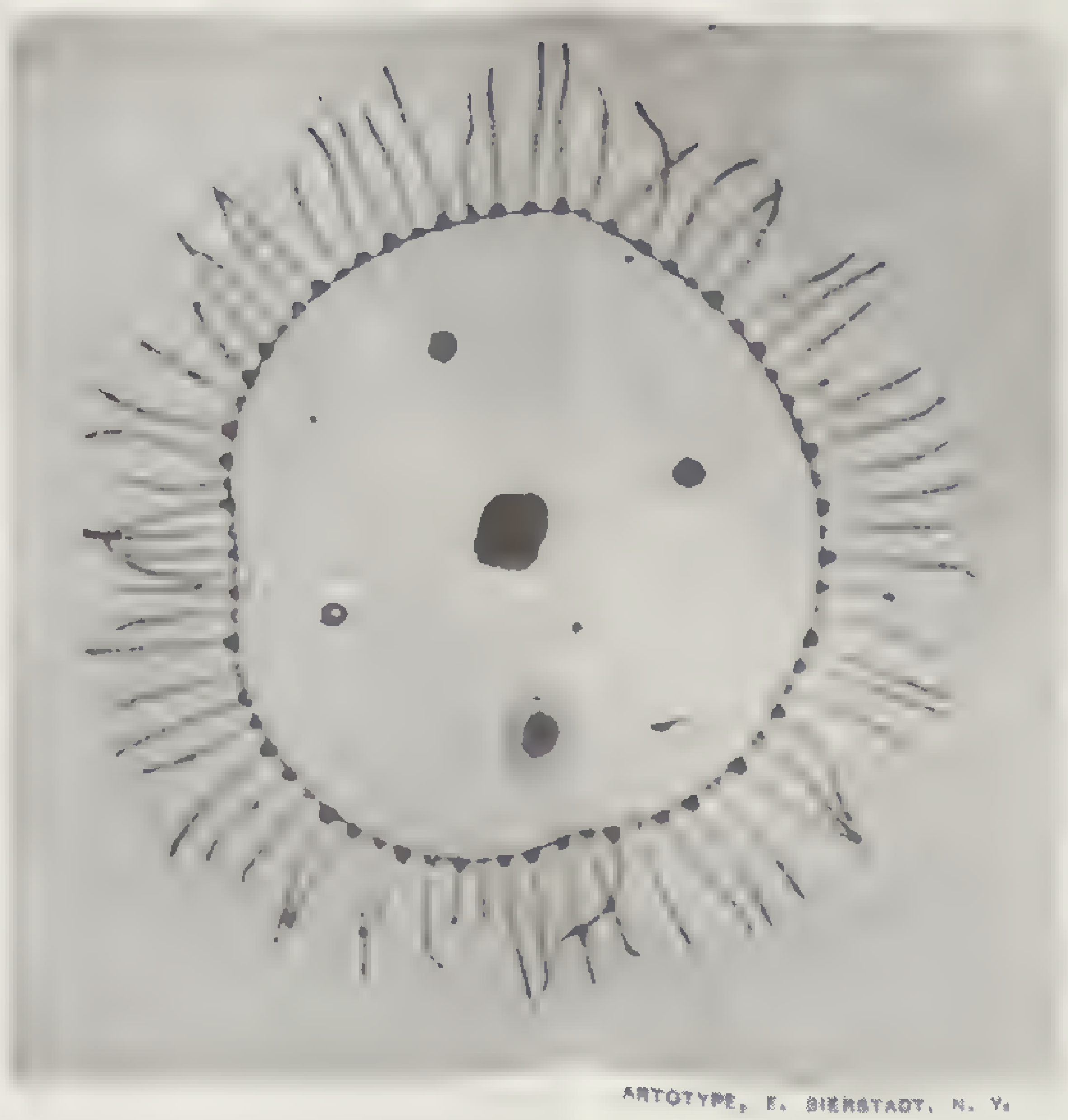

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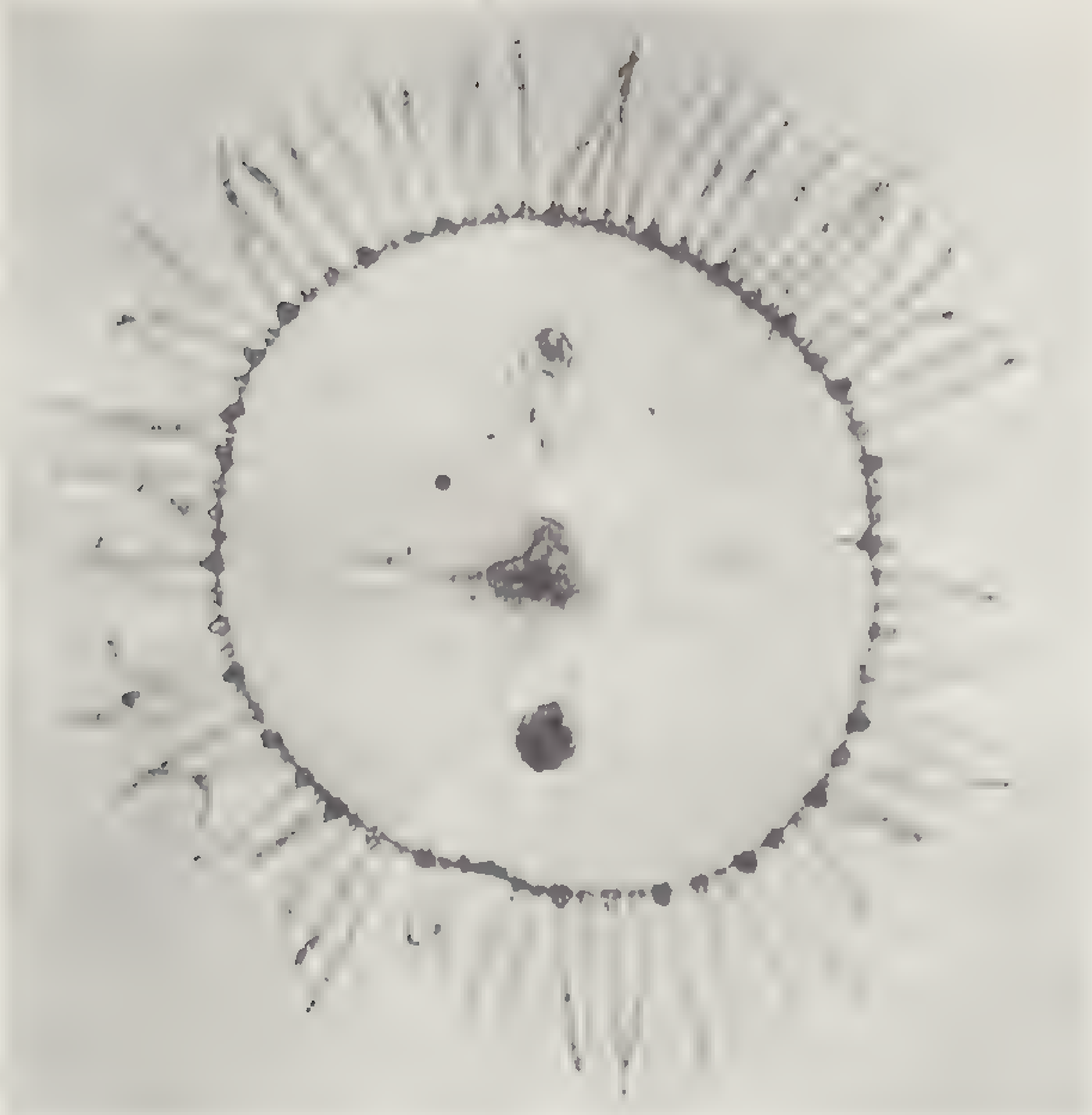

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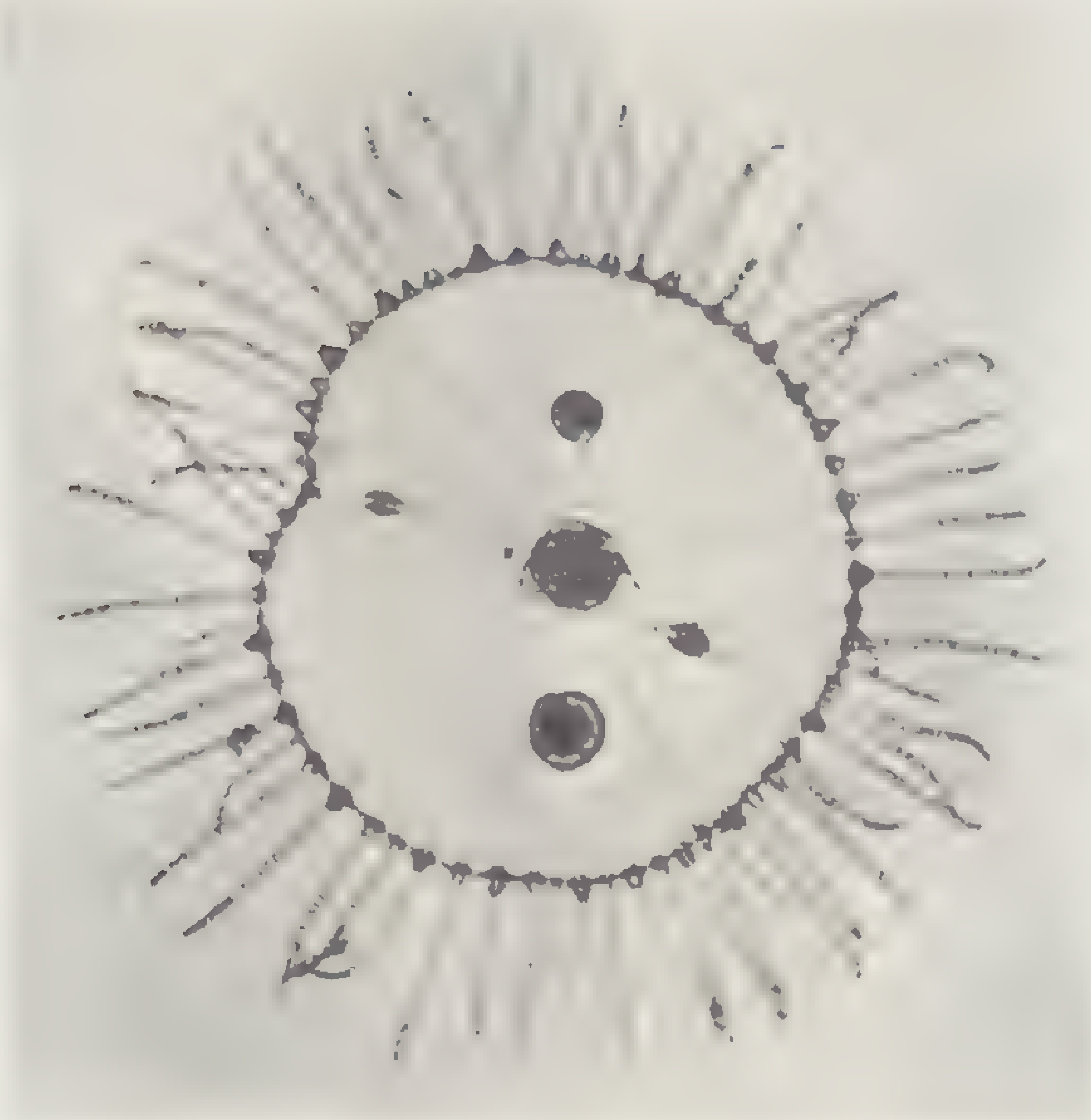

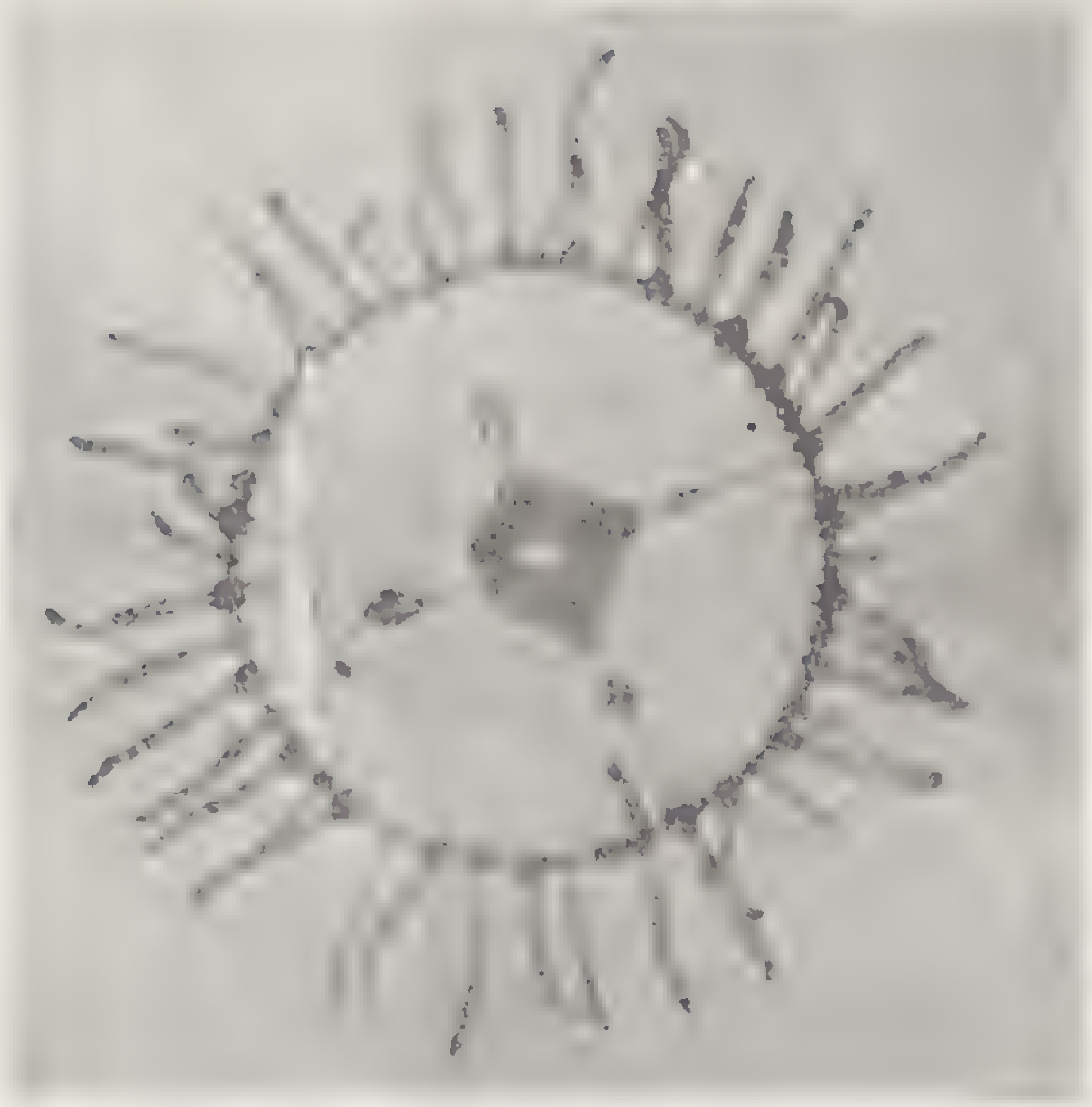

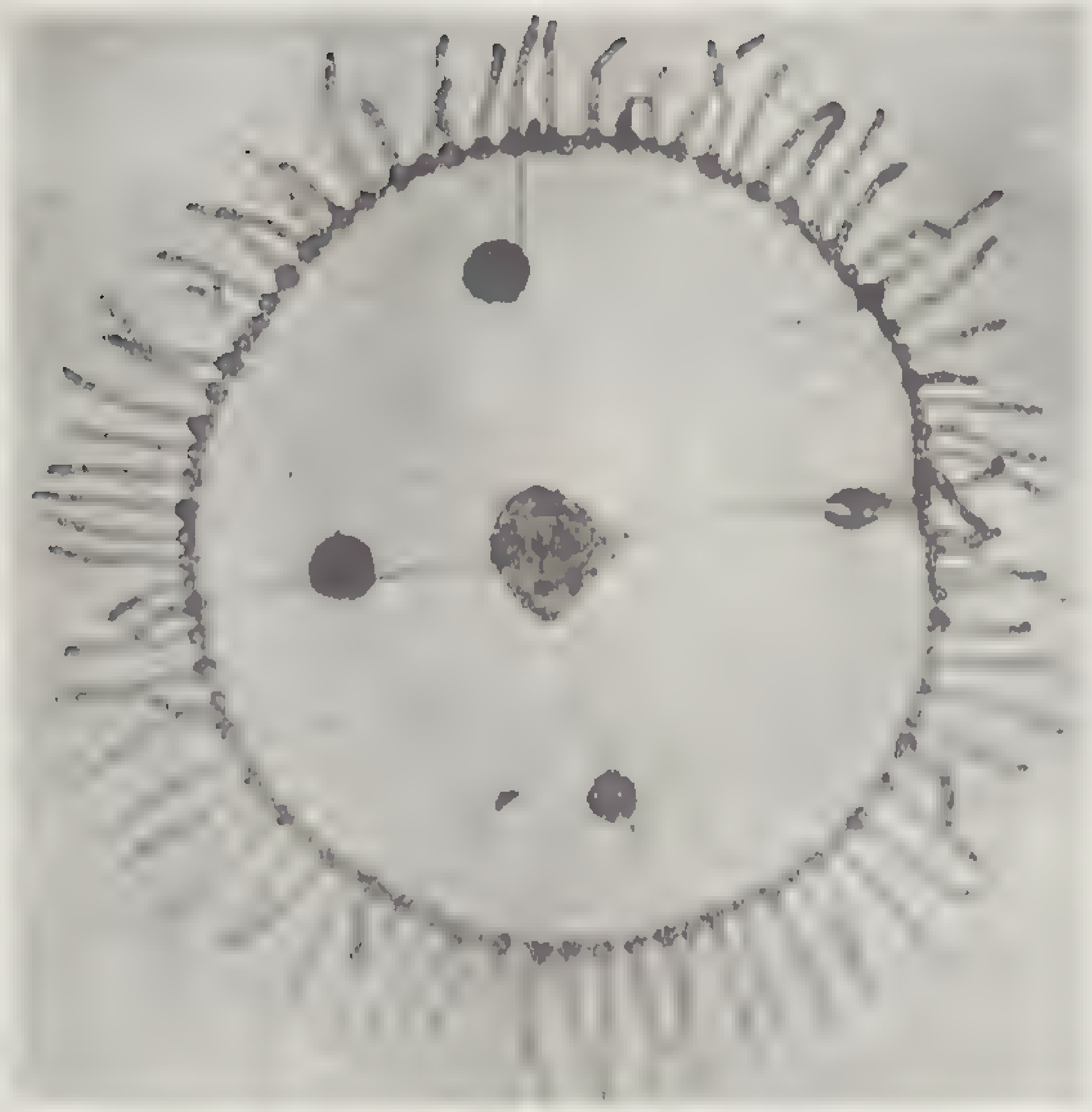

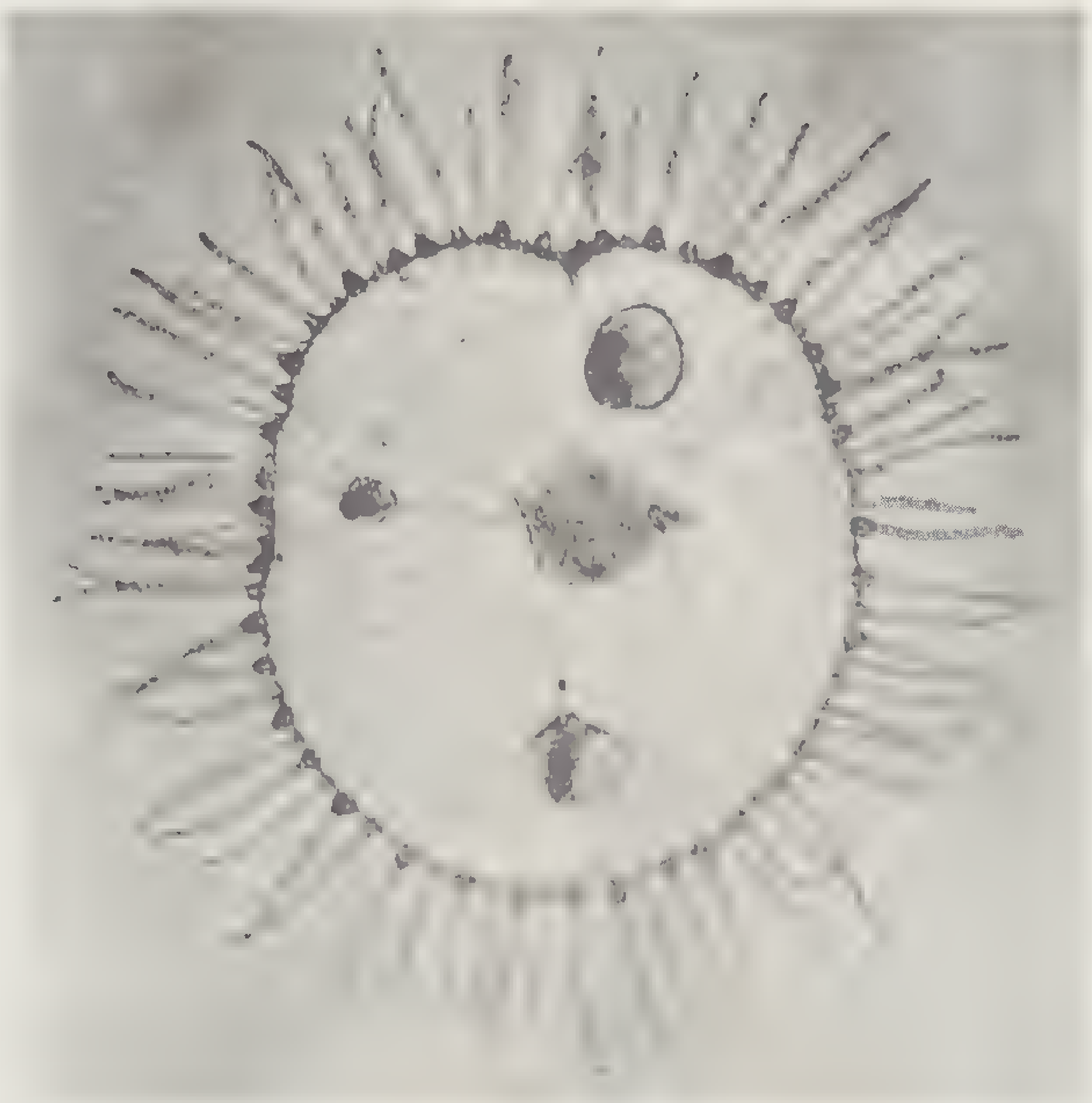

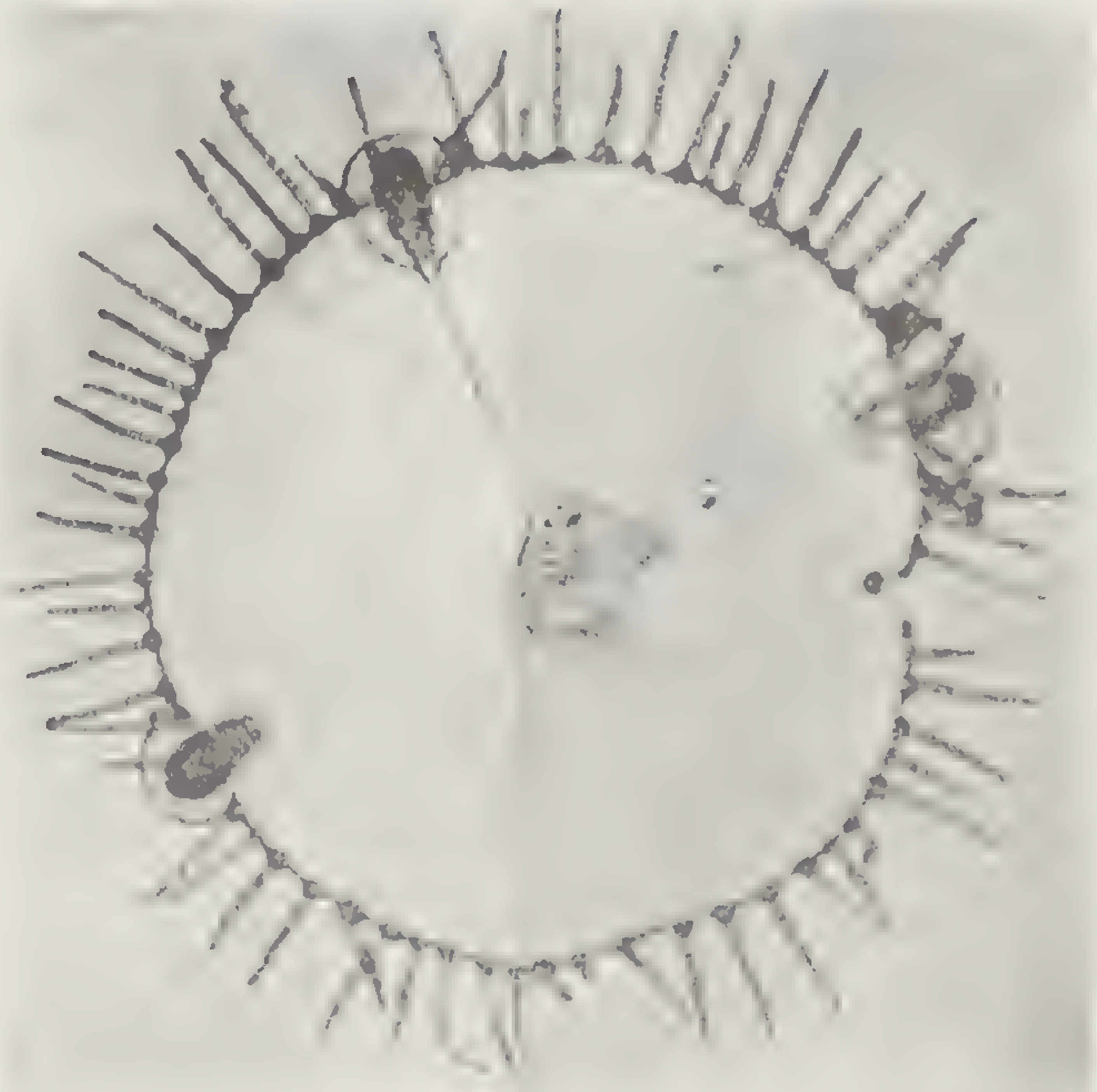

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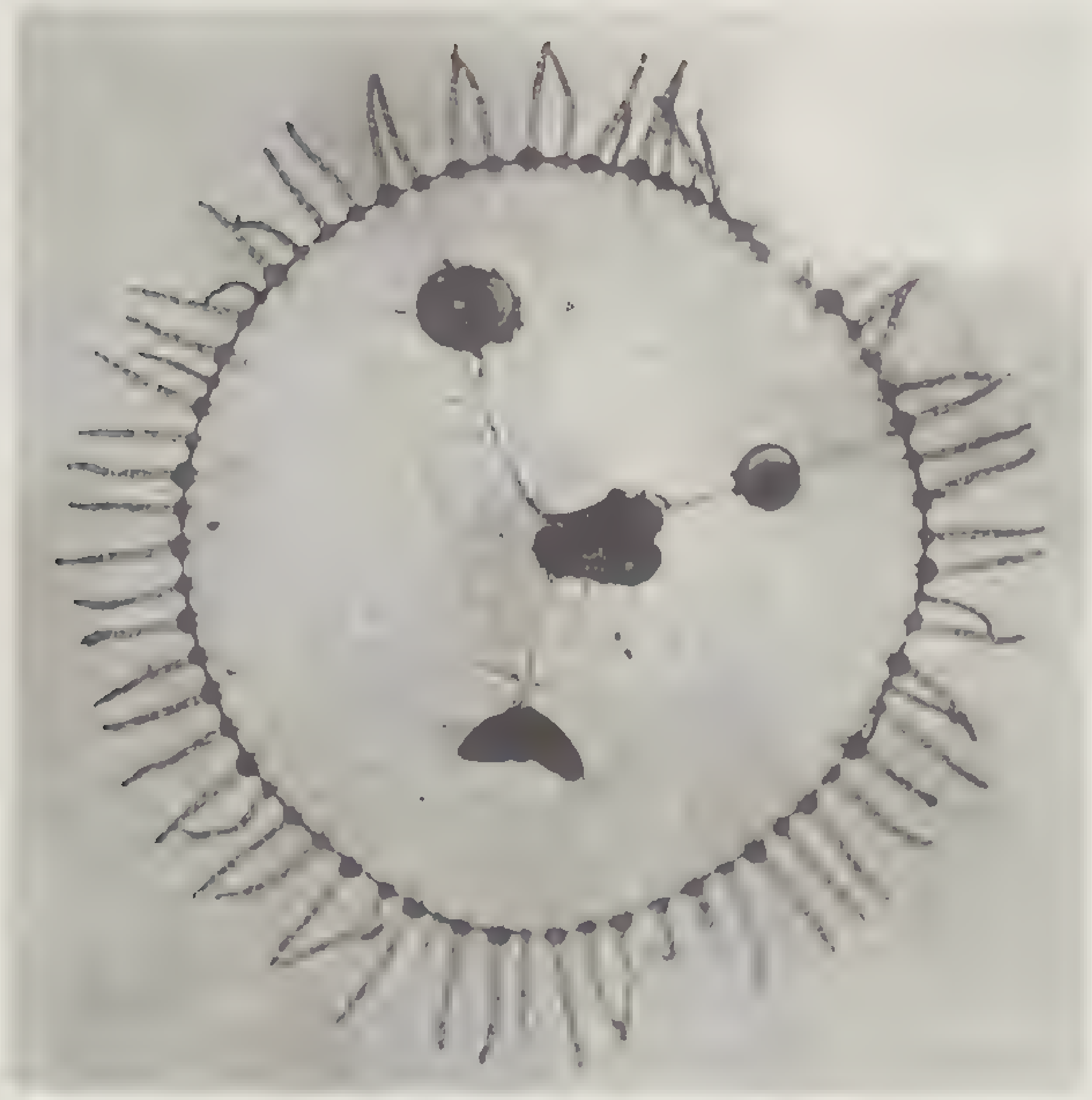

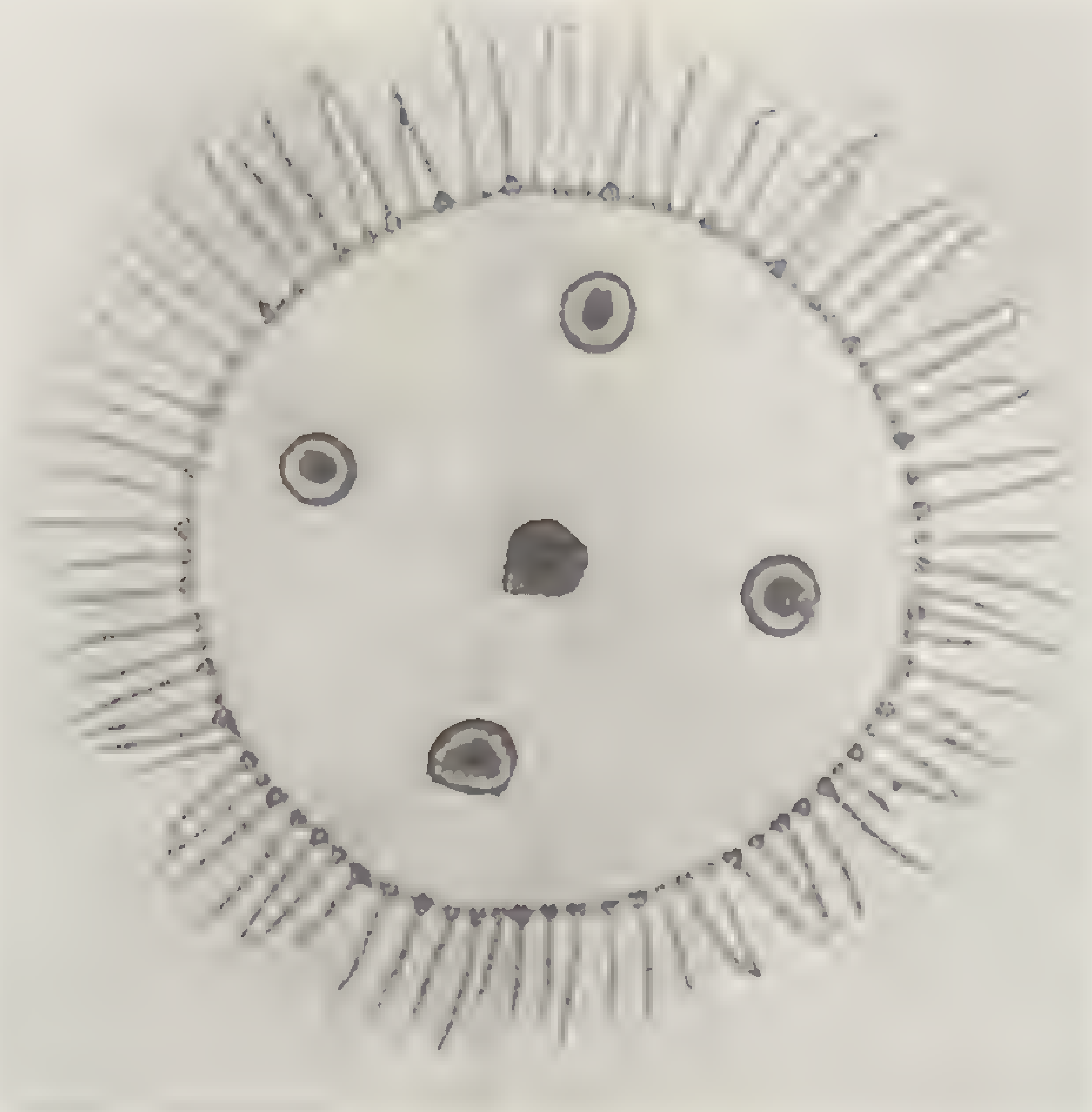

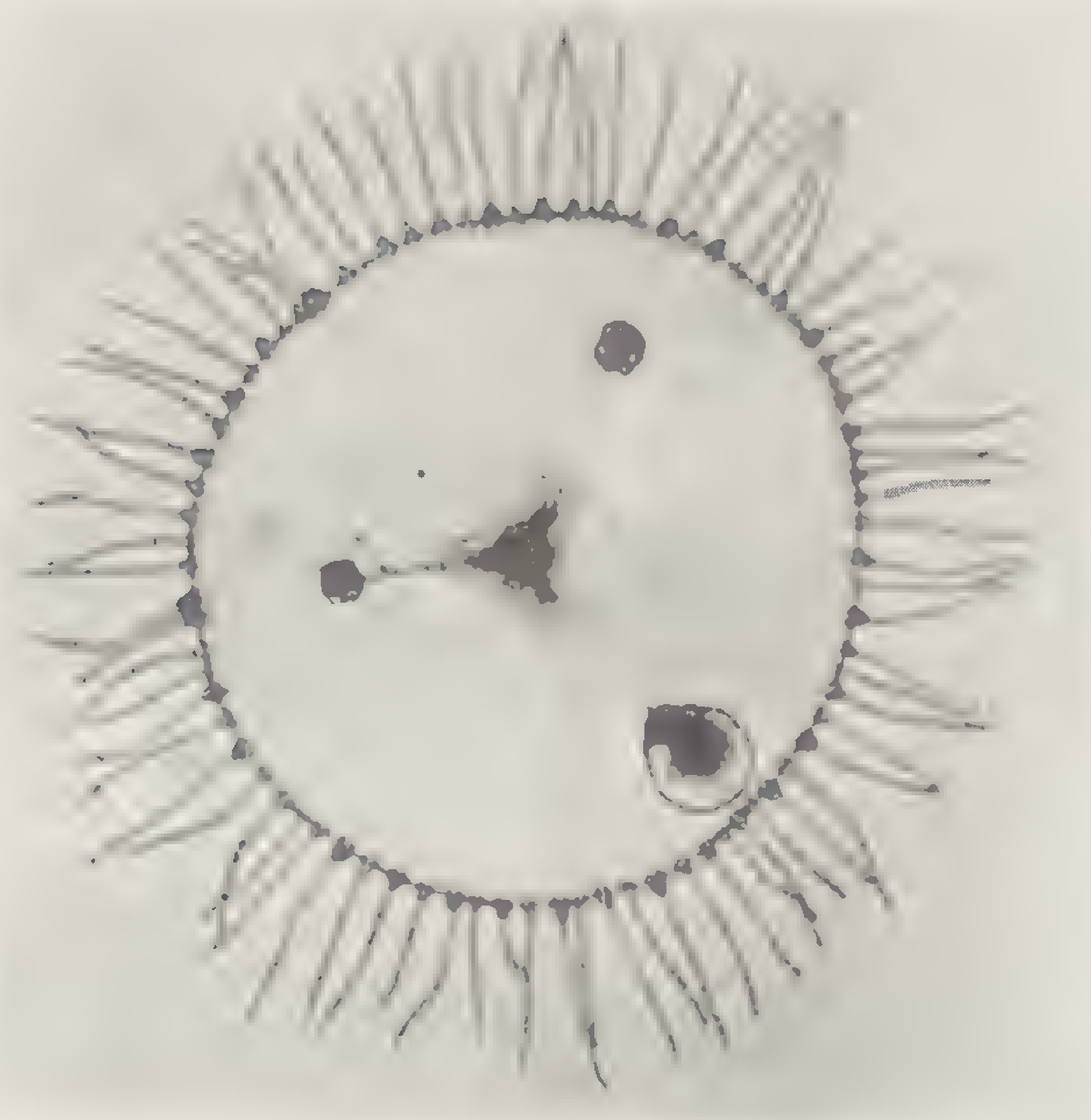

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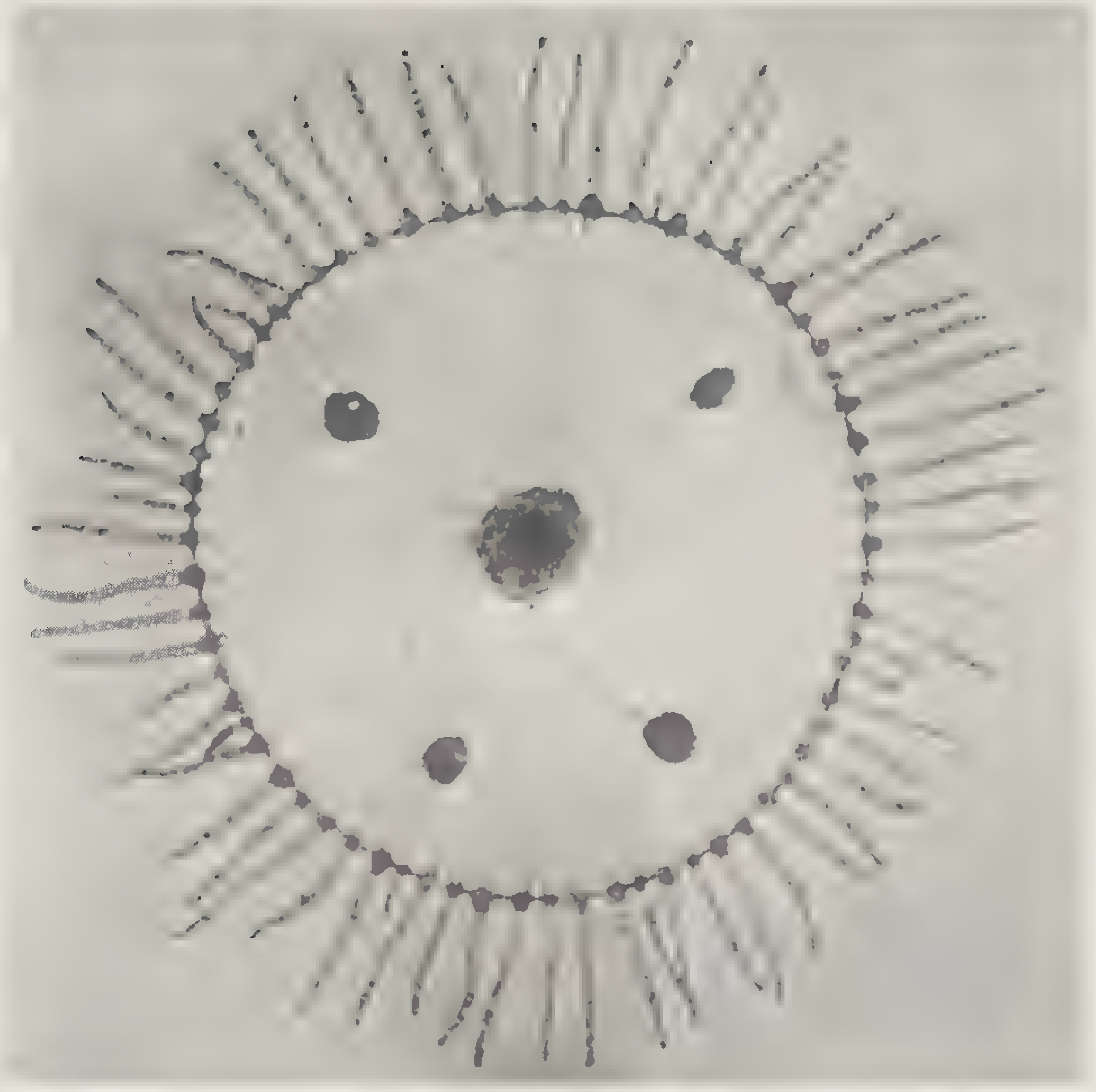

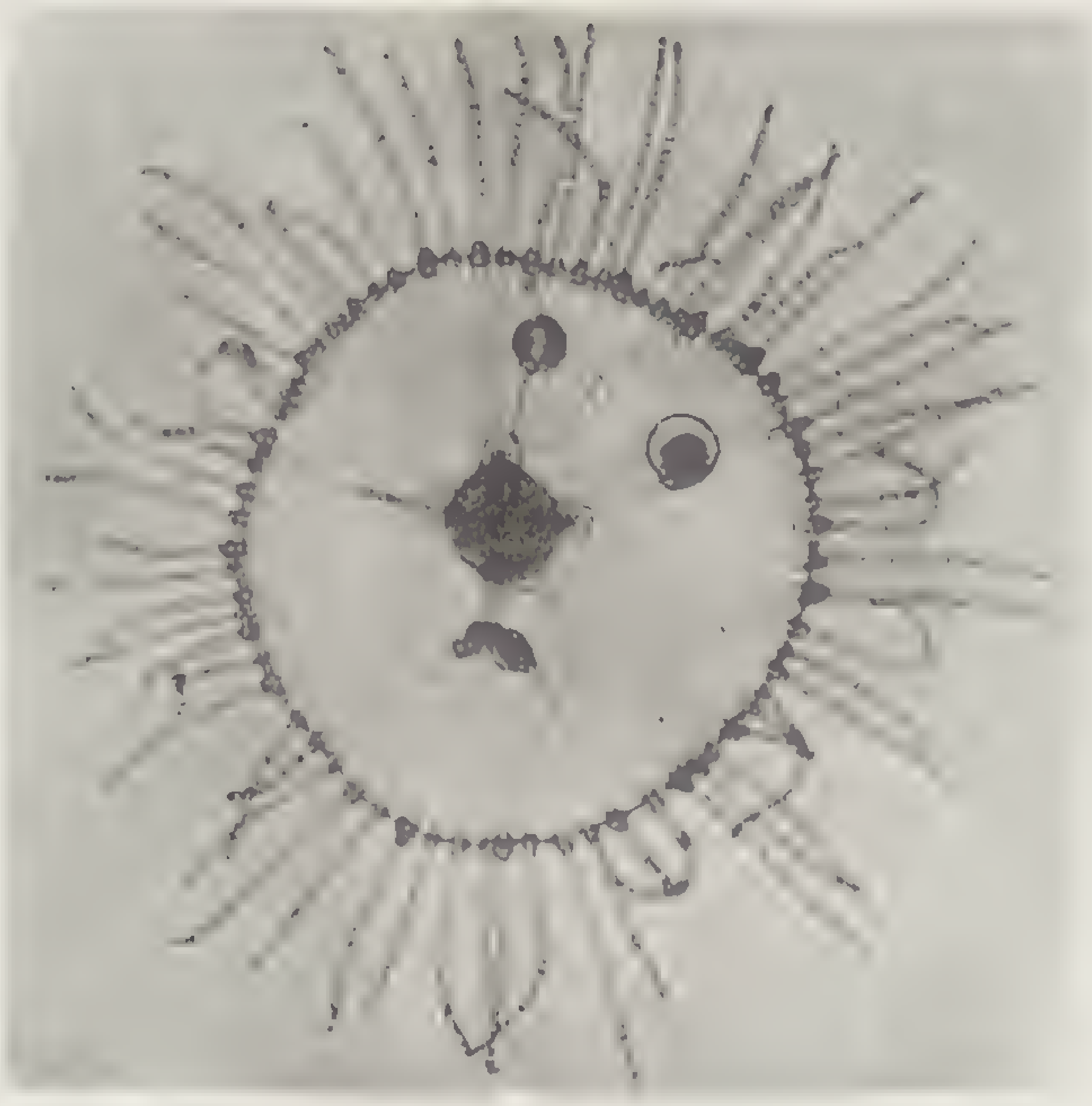

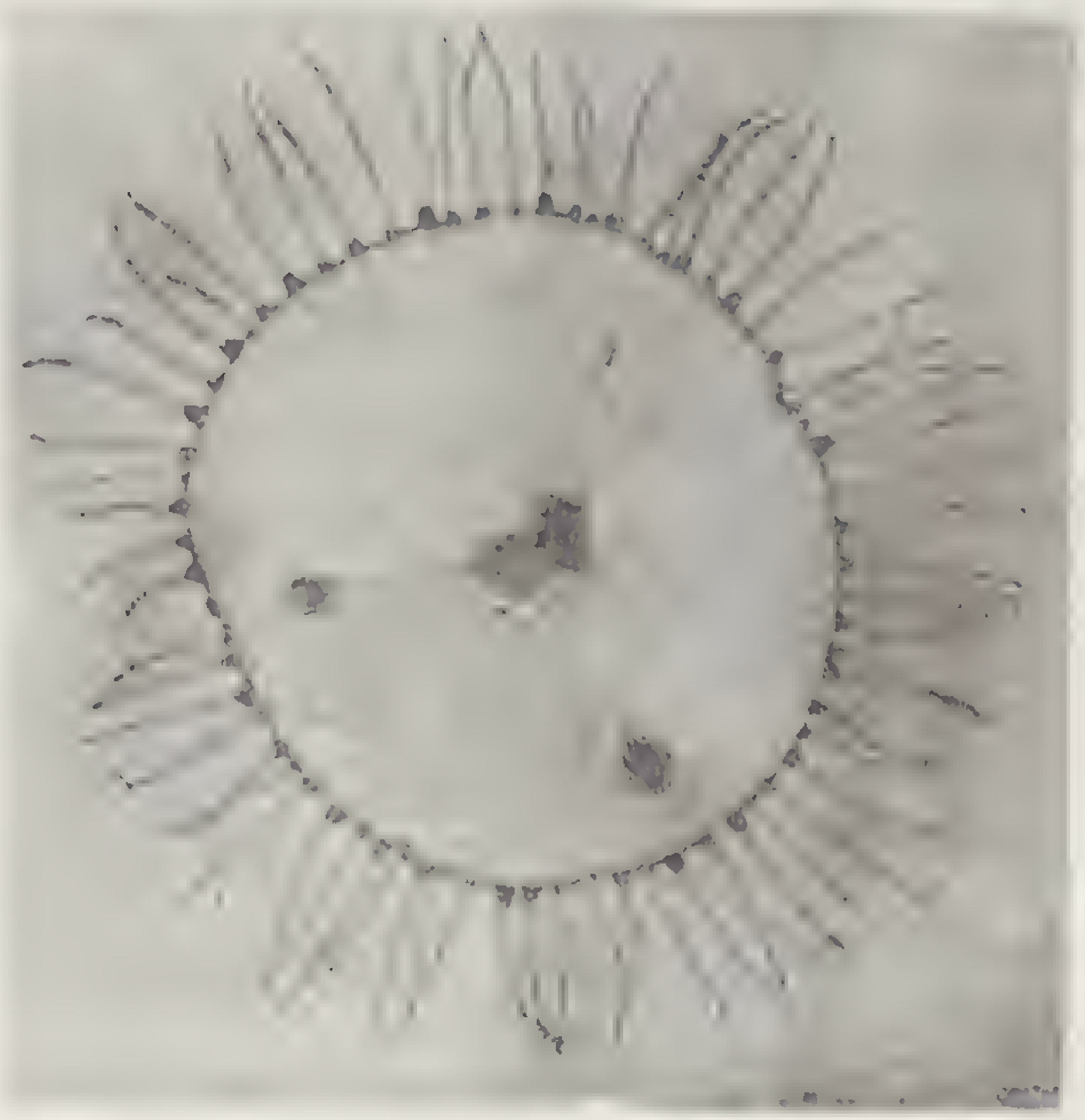

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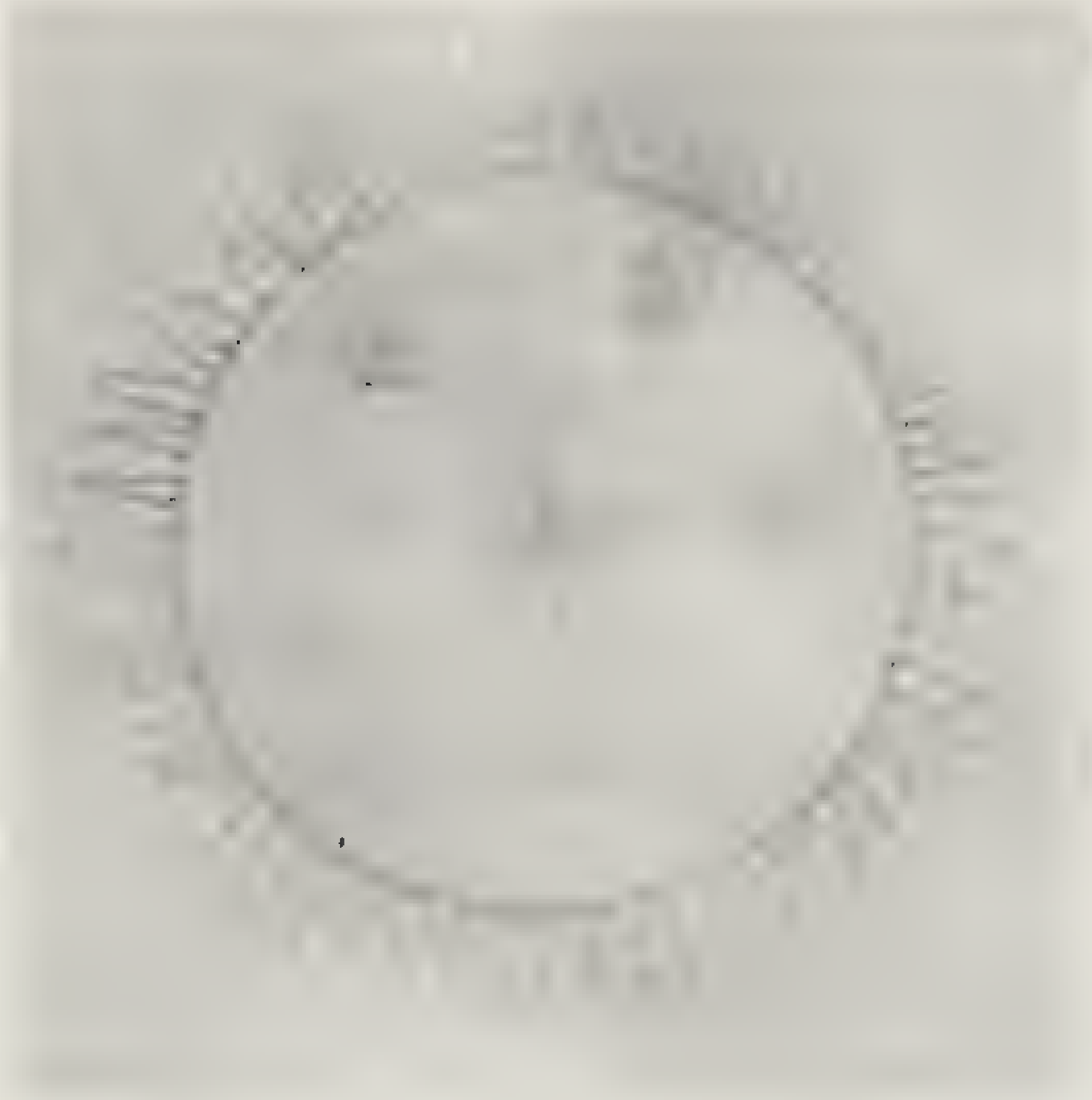
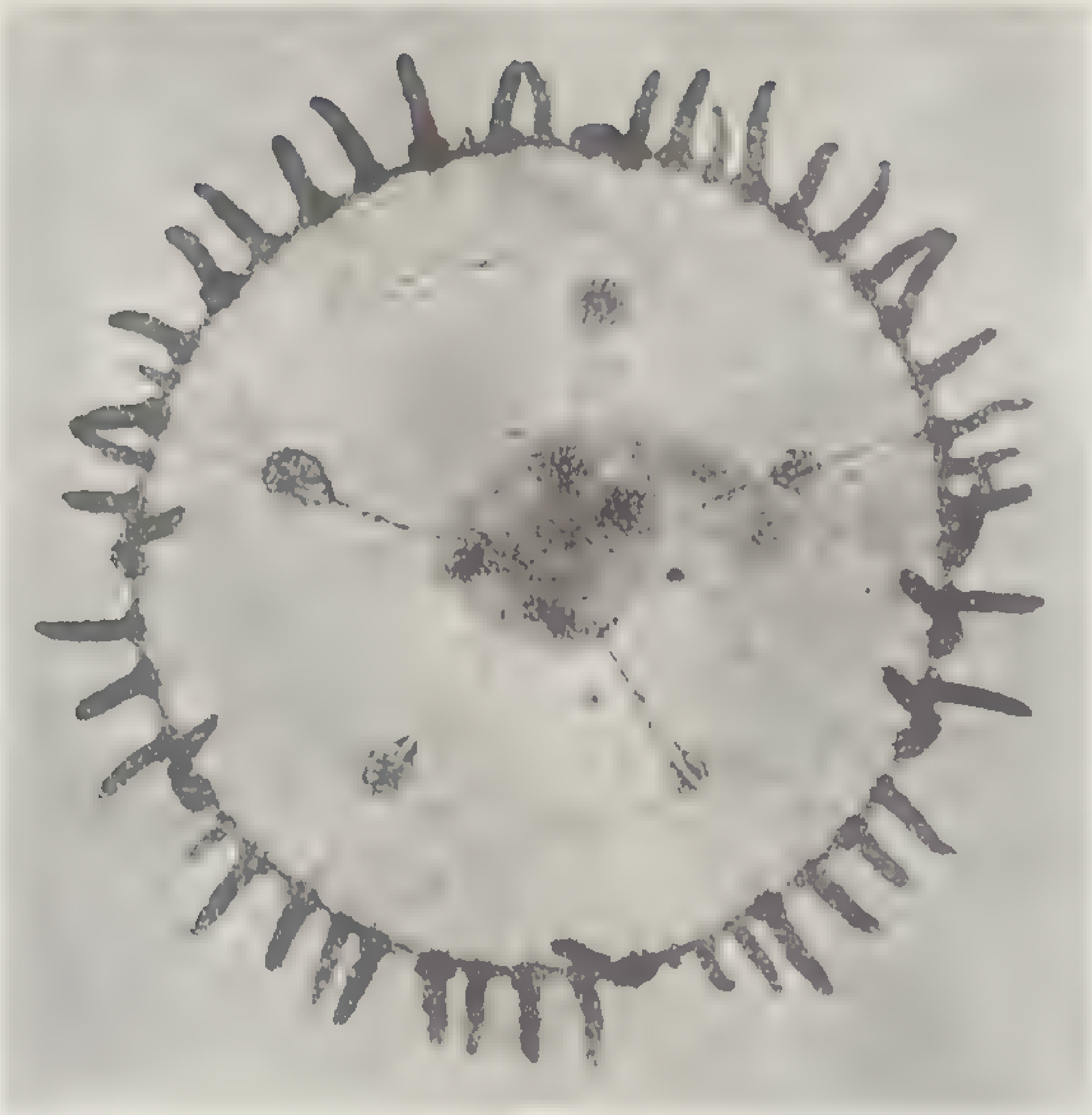
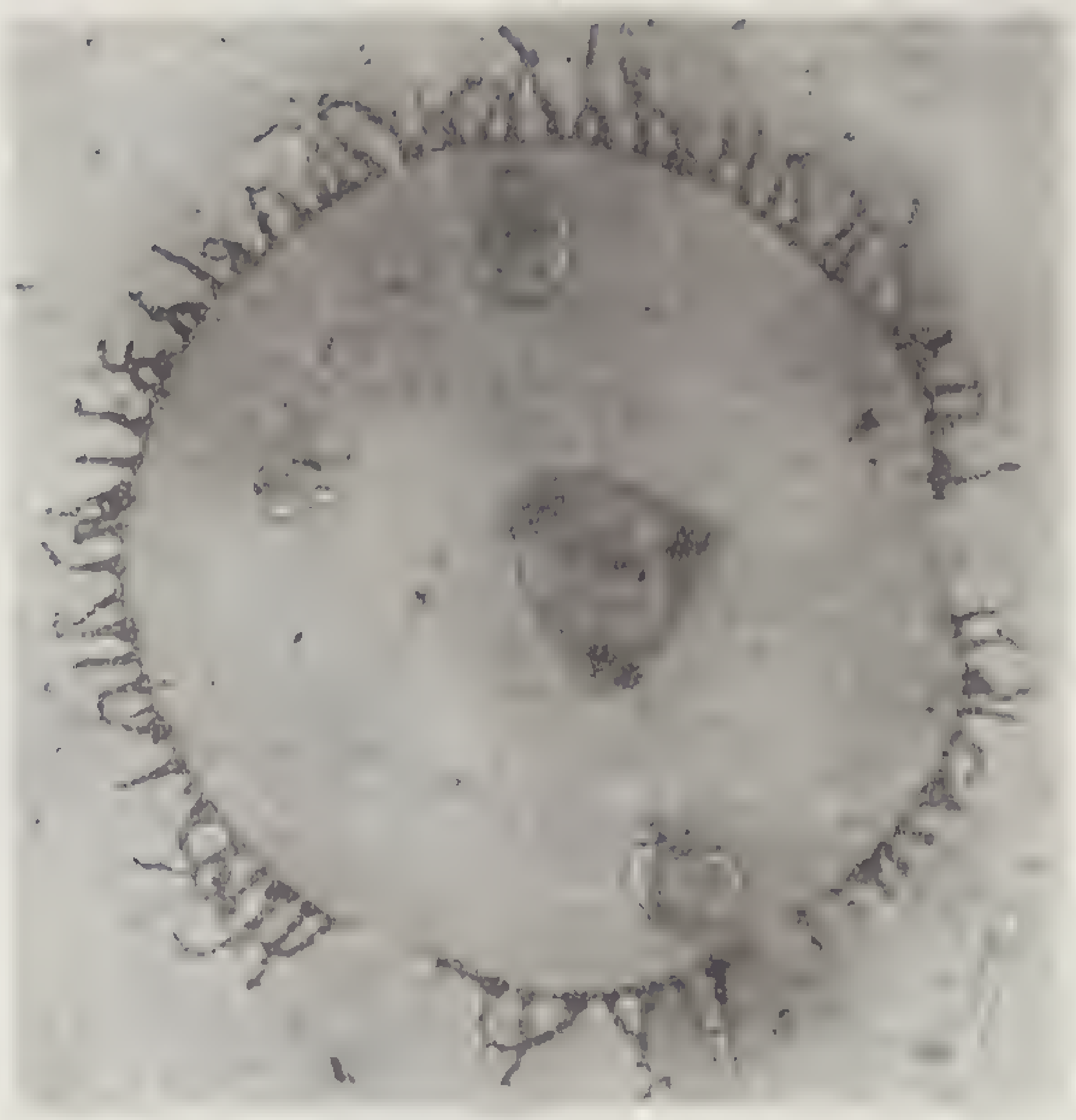
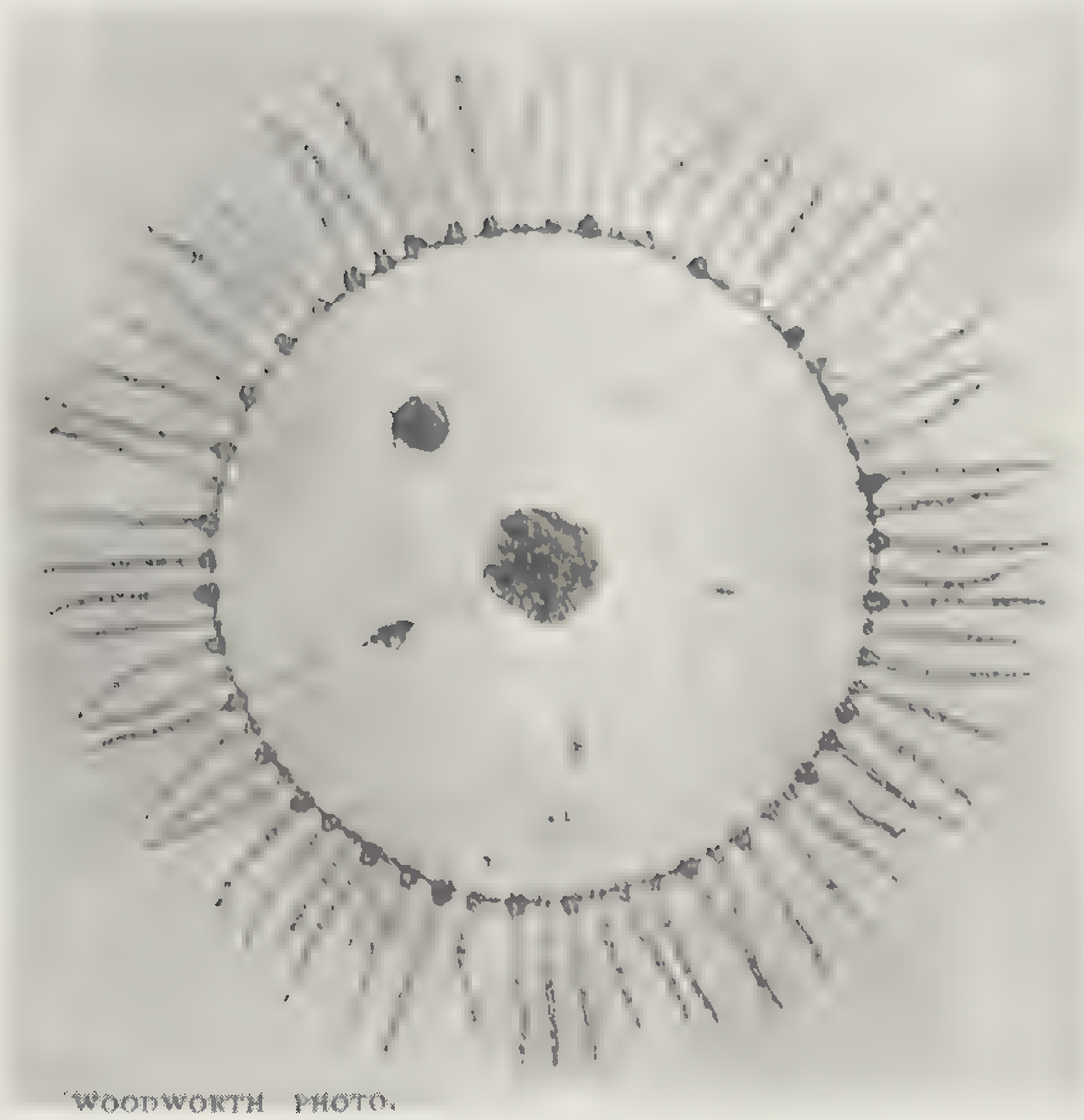
EUCOPE

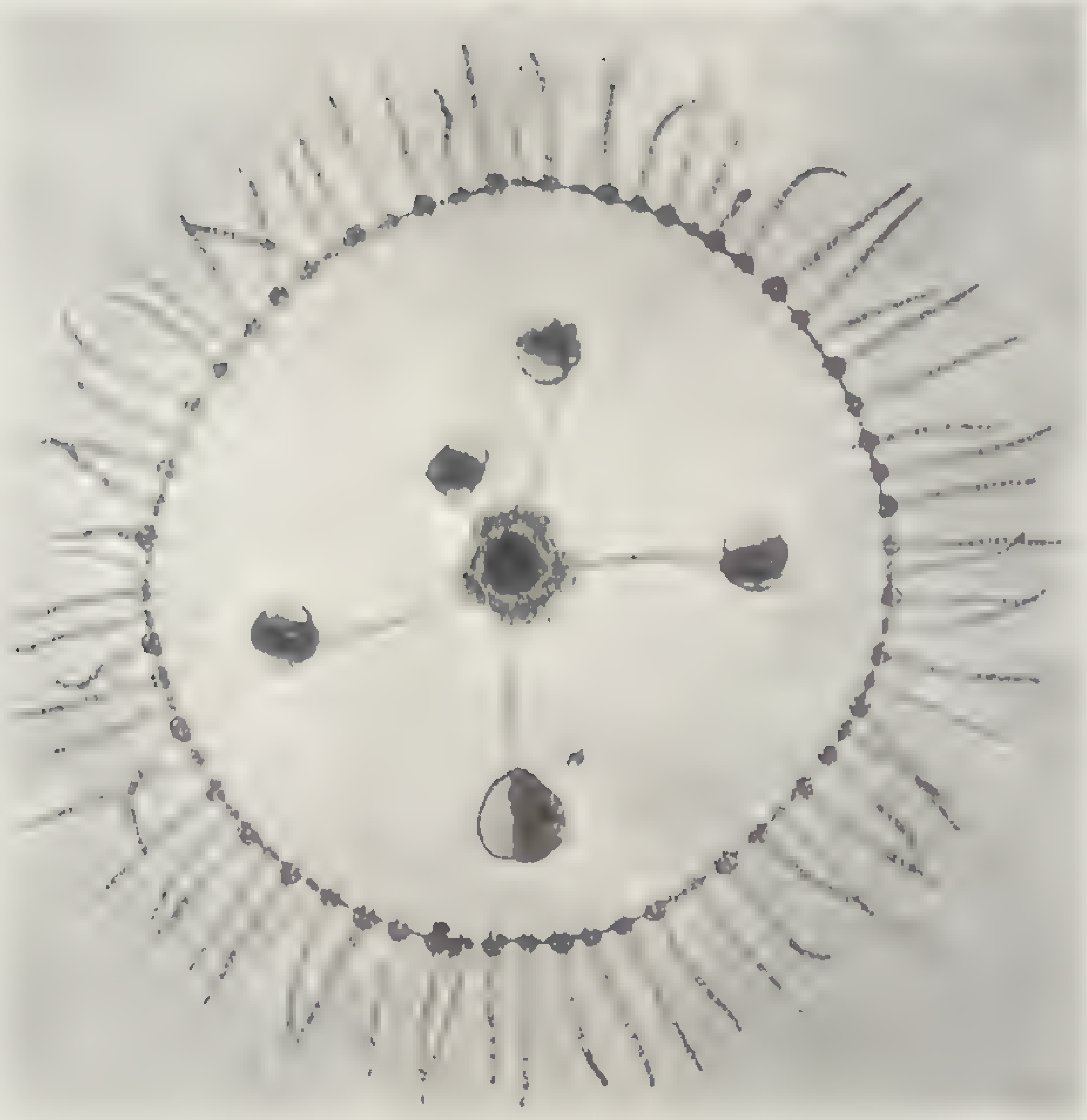
PLATE IV.

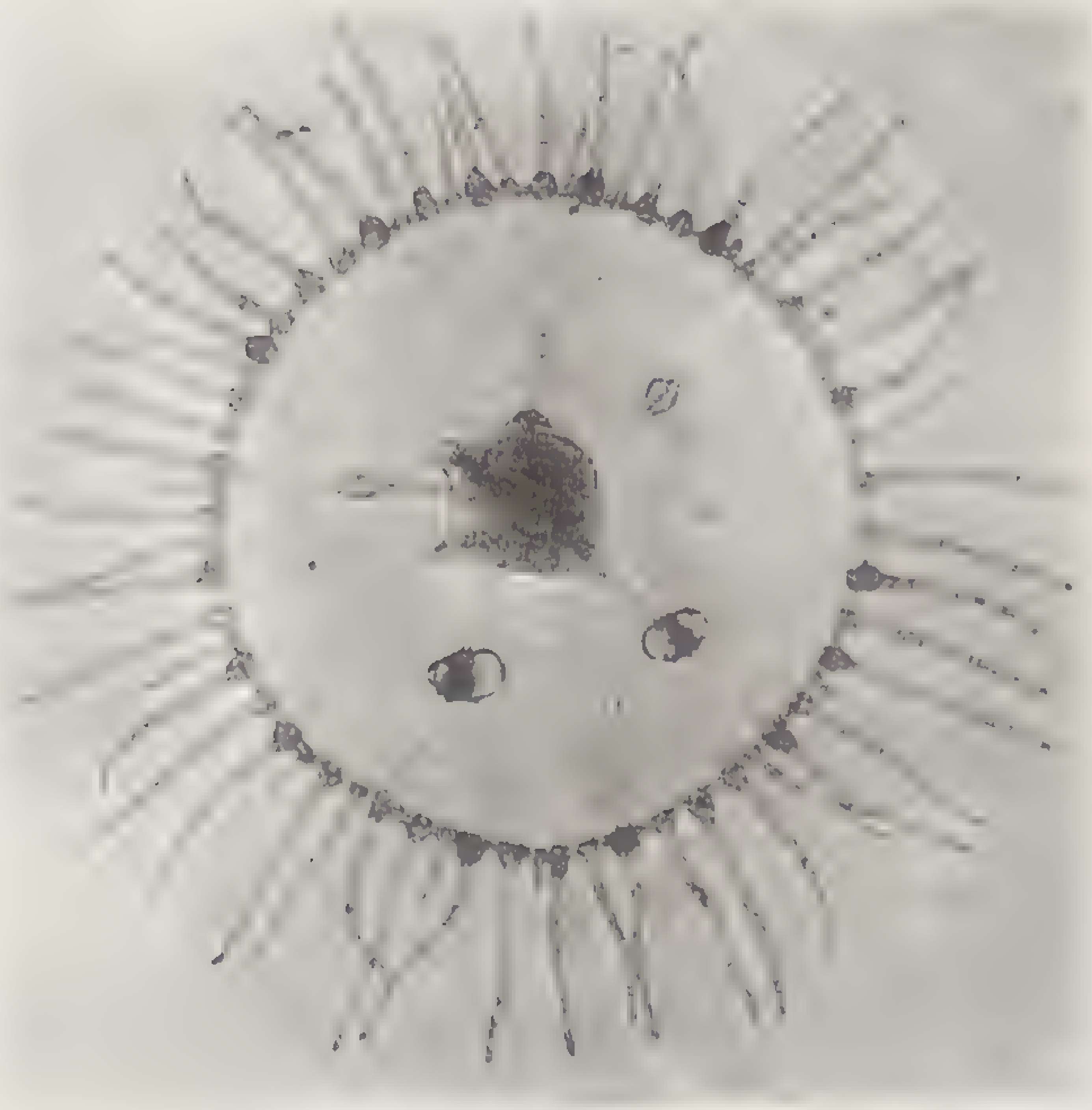












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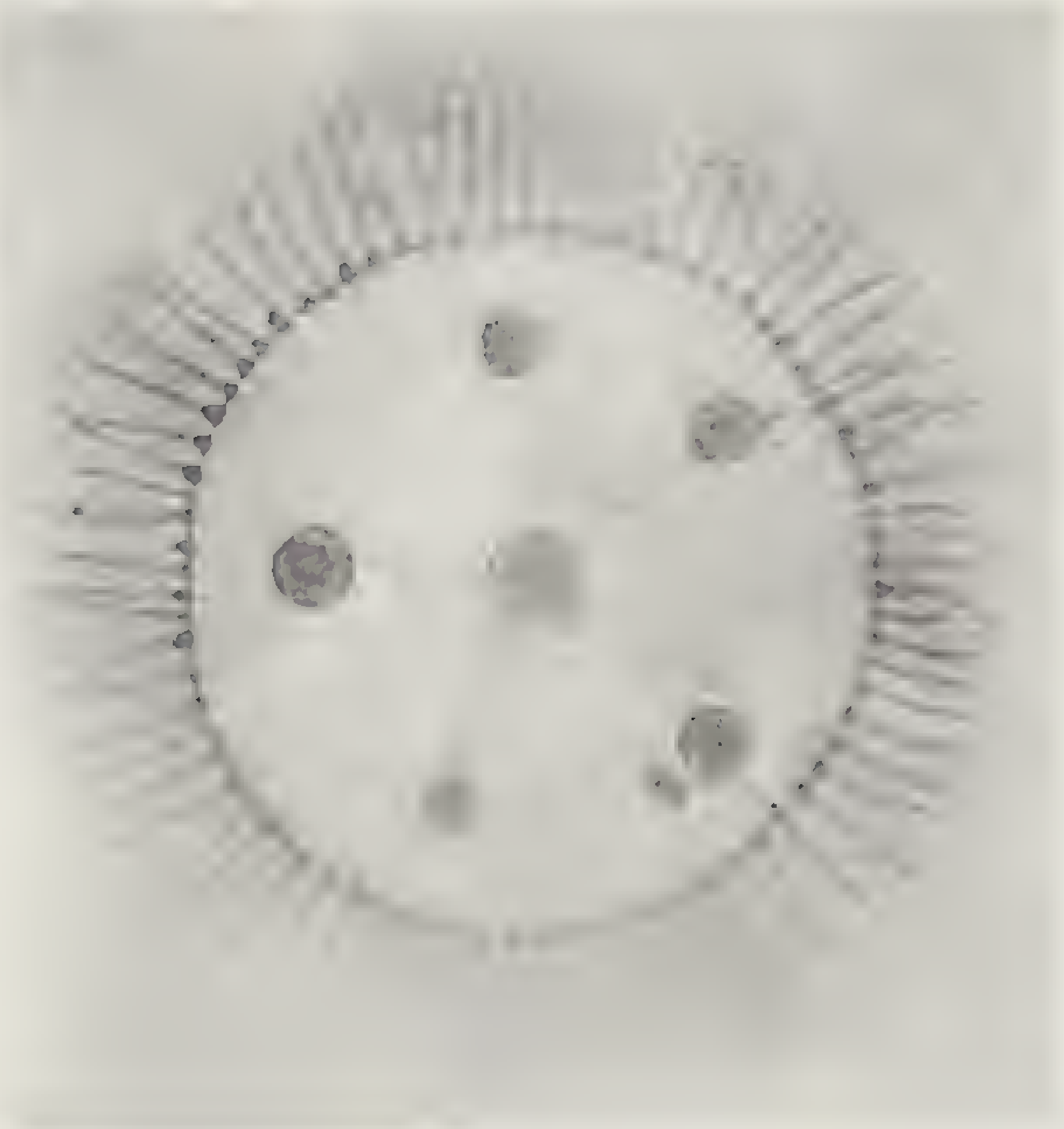

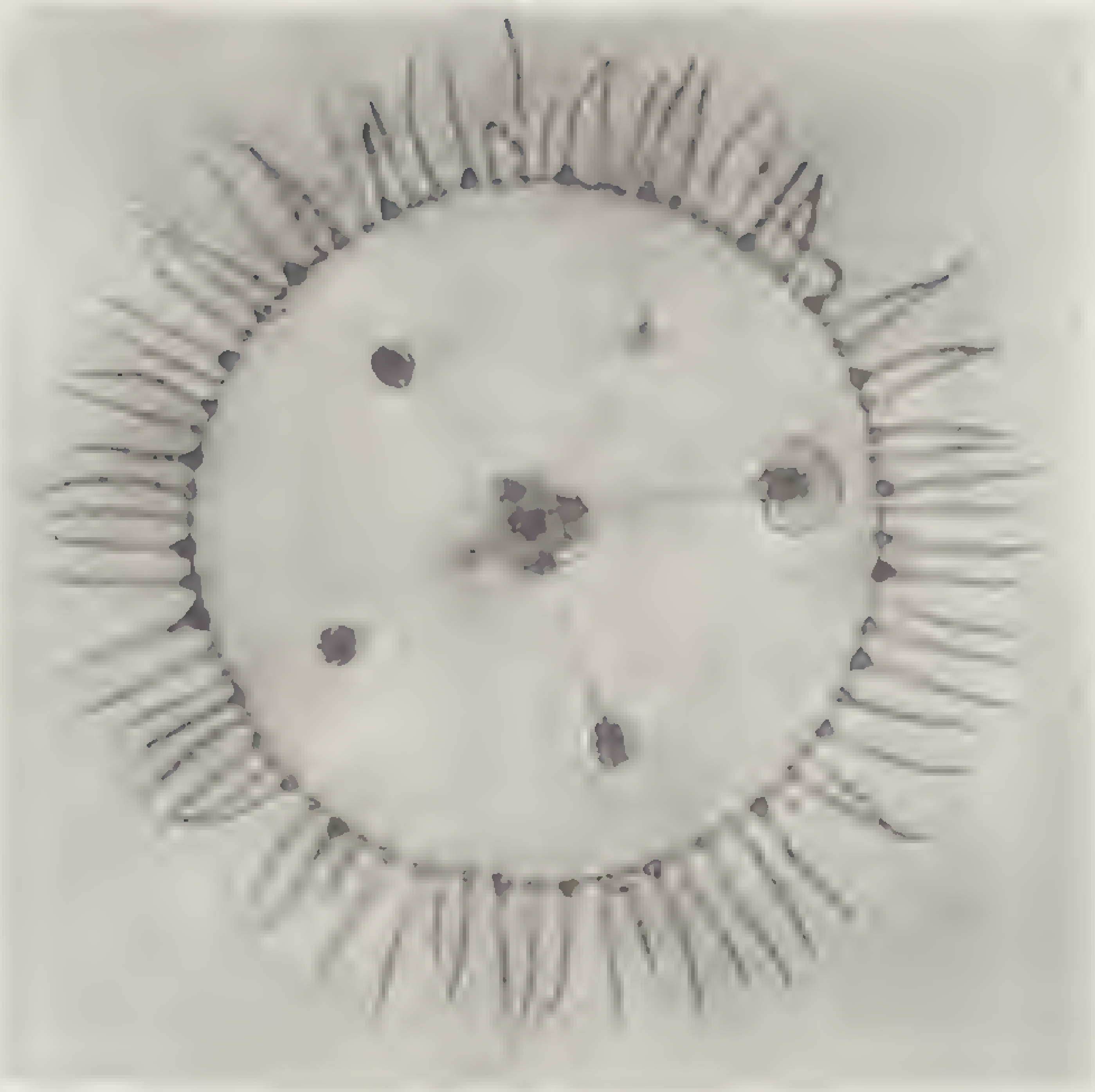

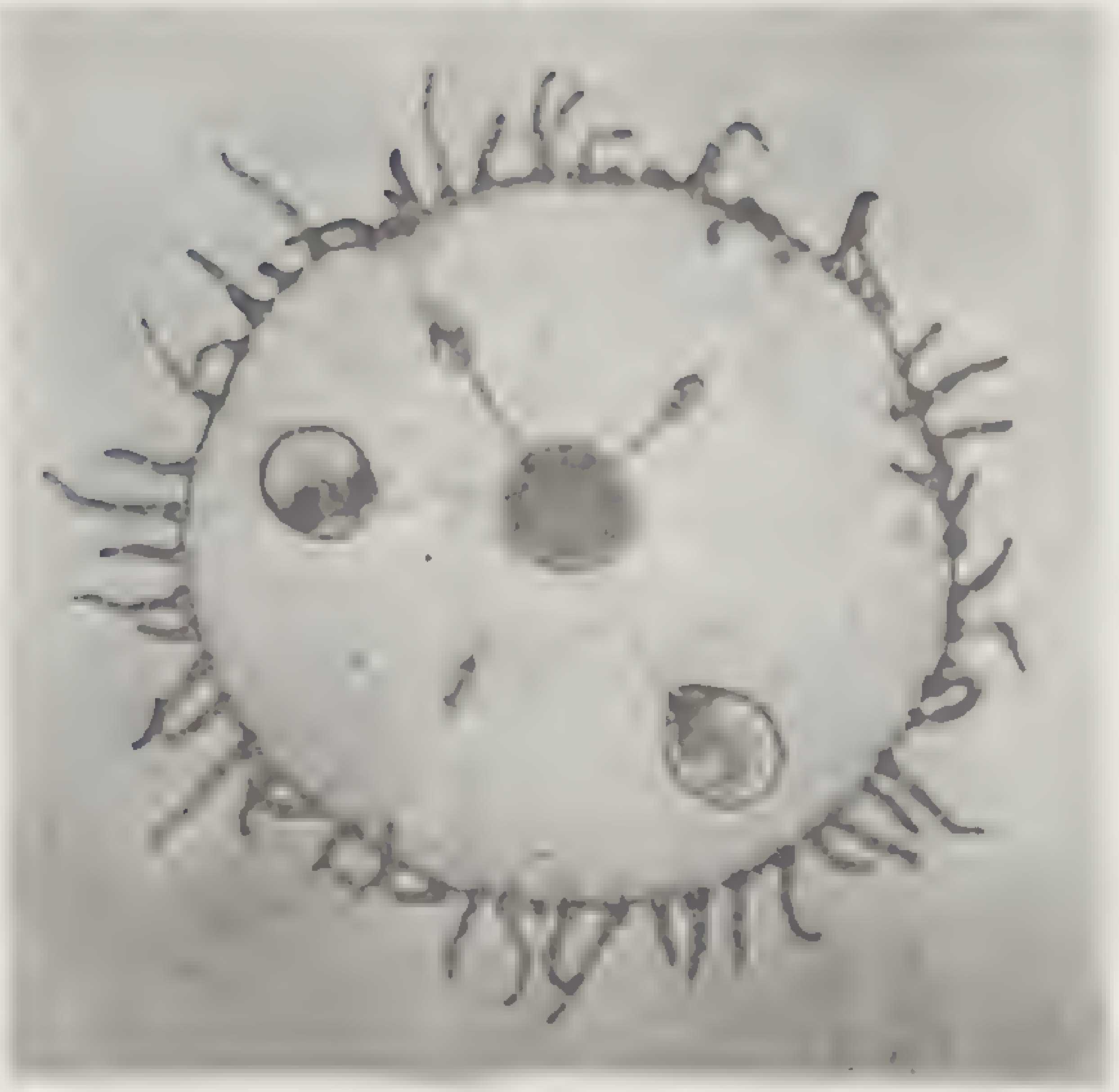

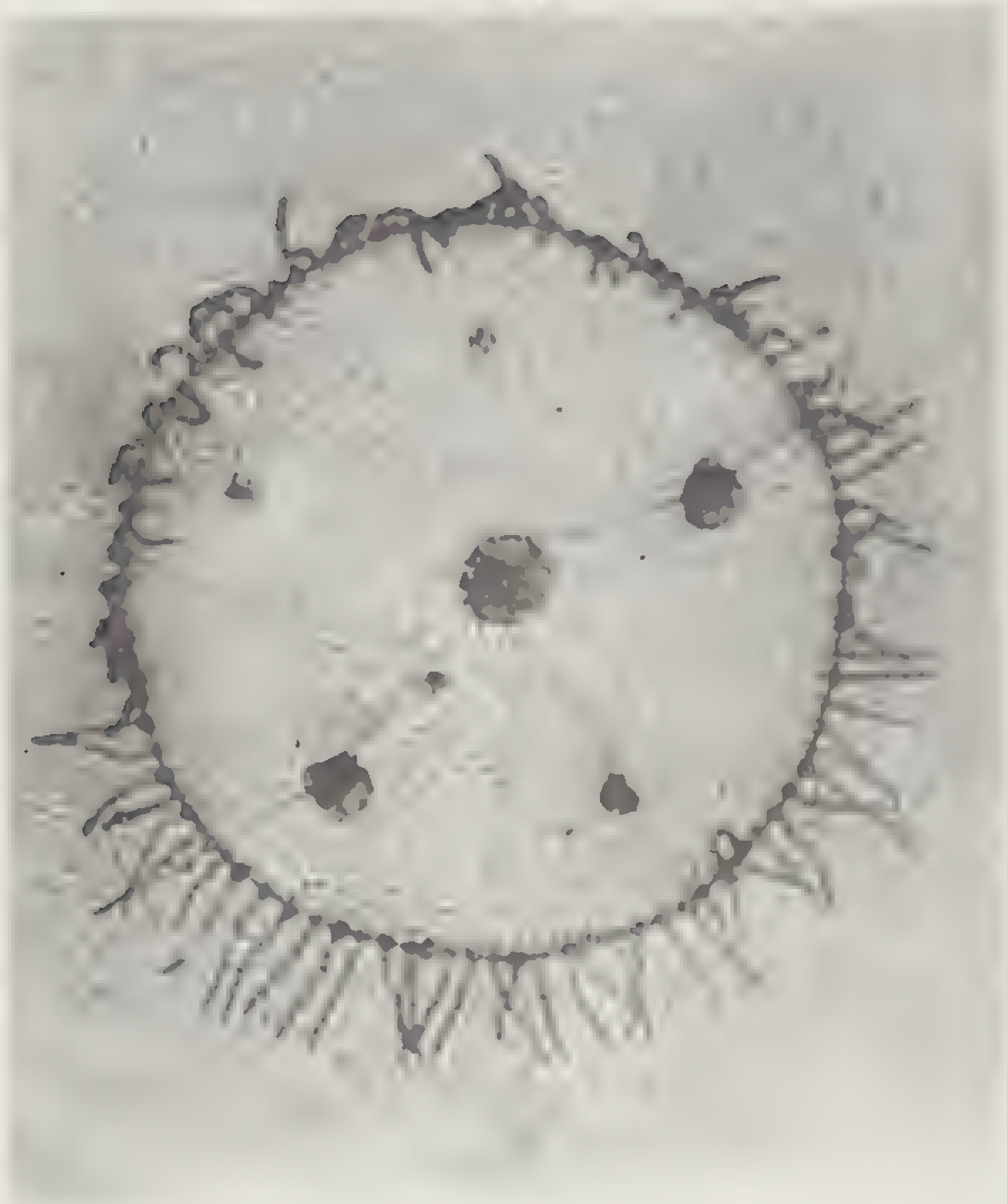

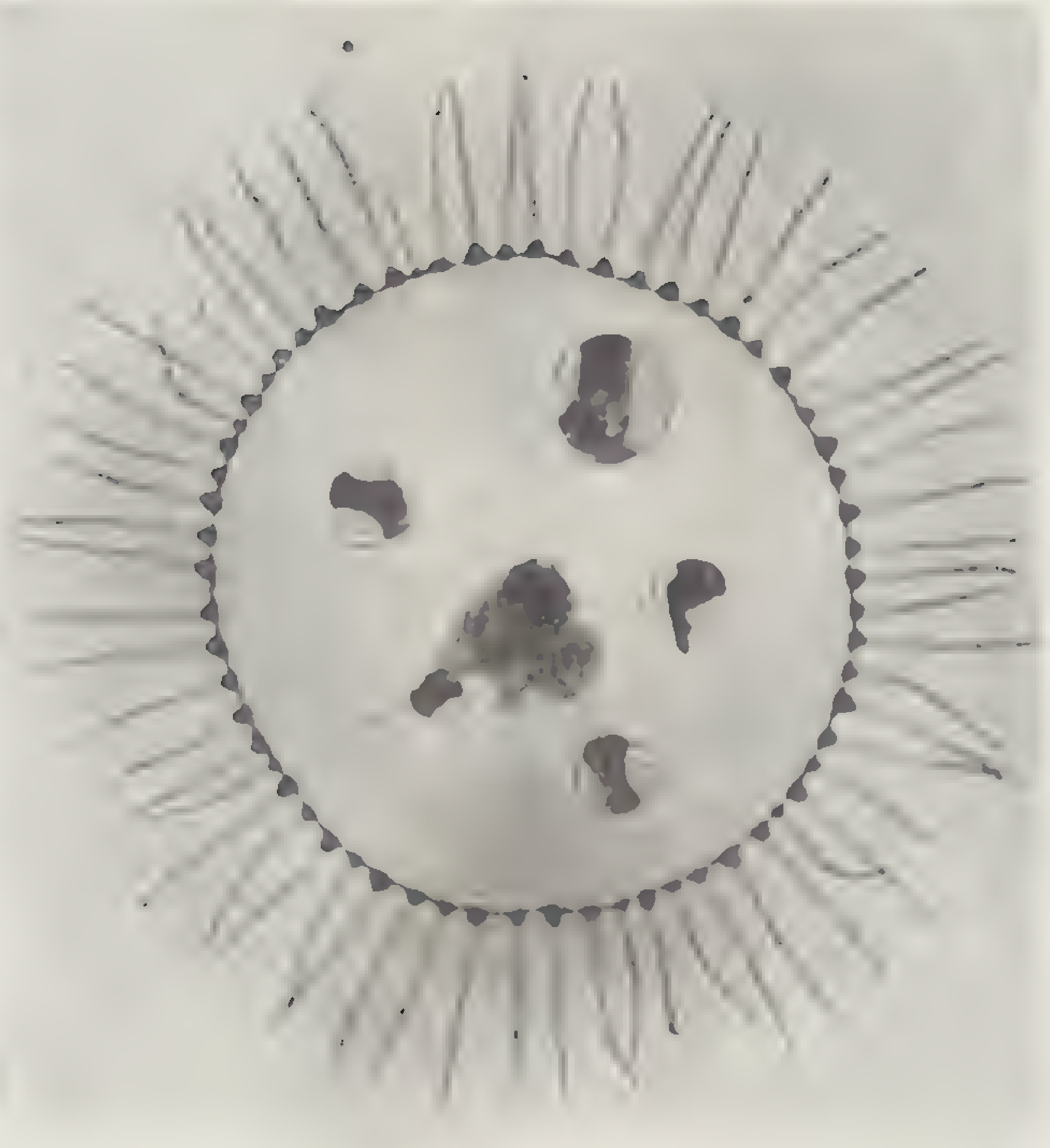

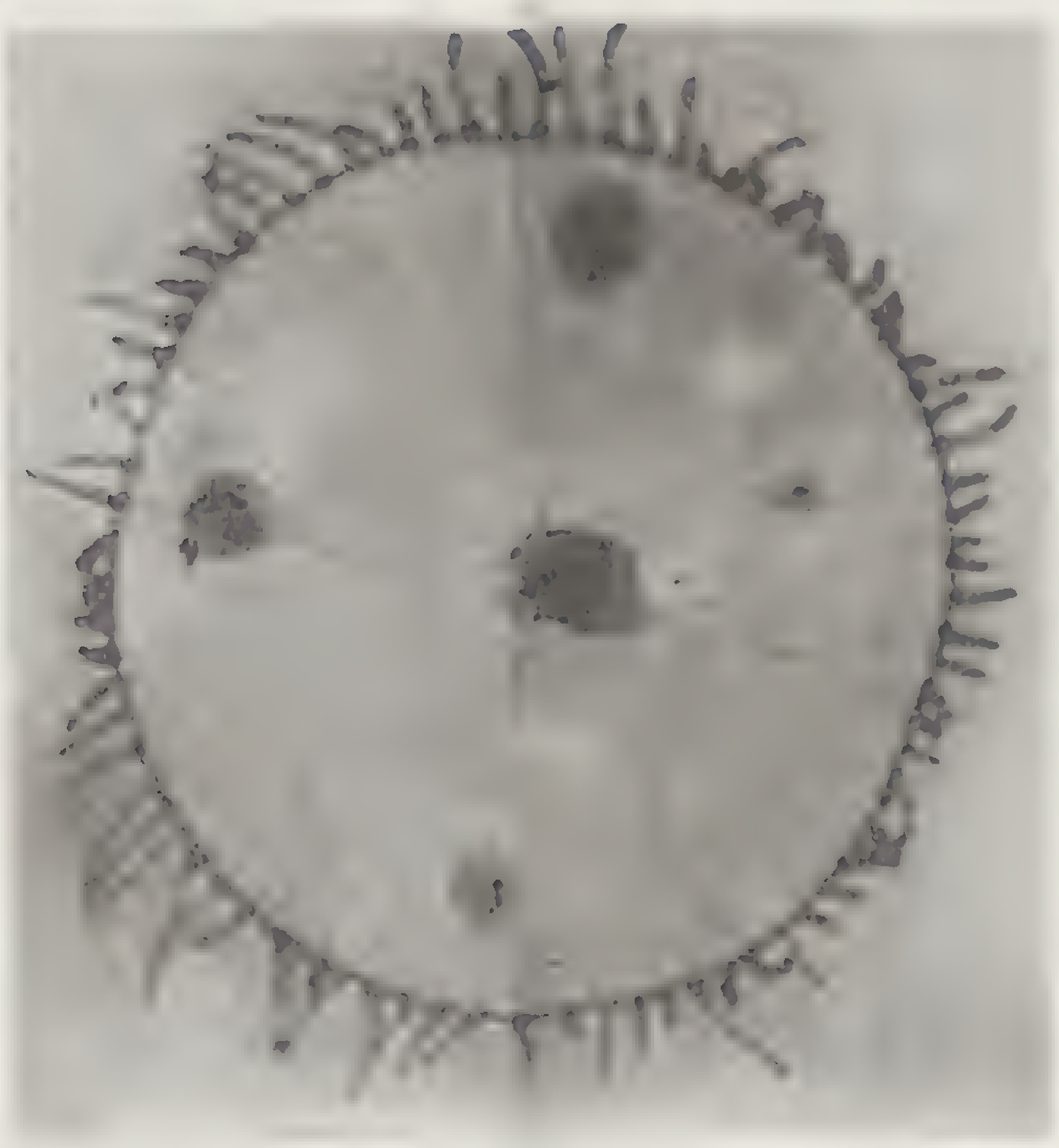

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PLATE VI.

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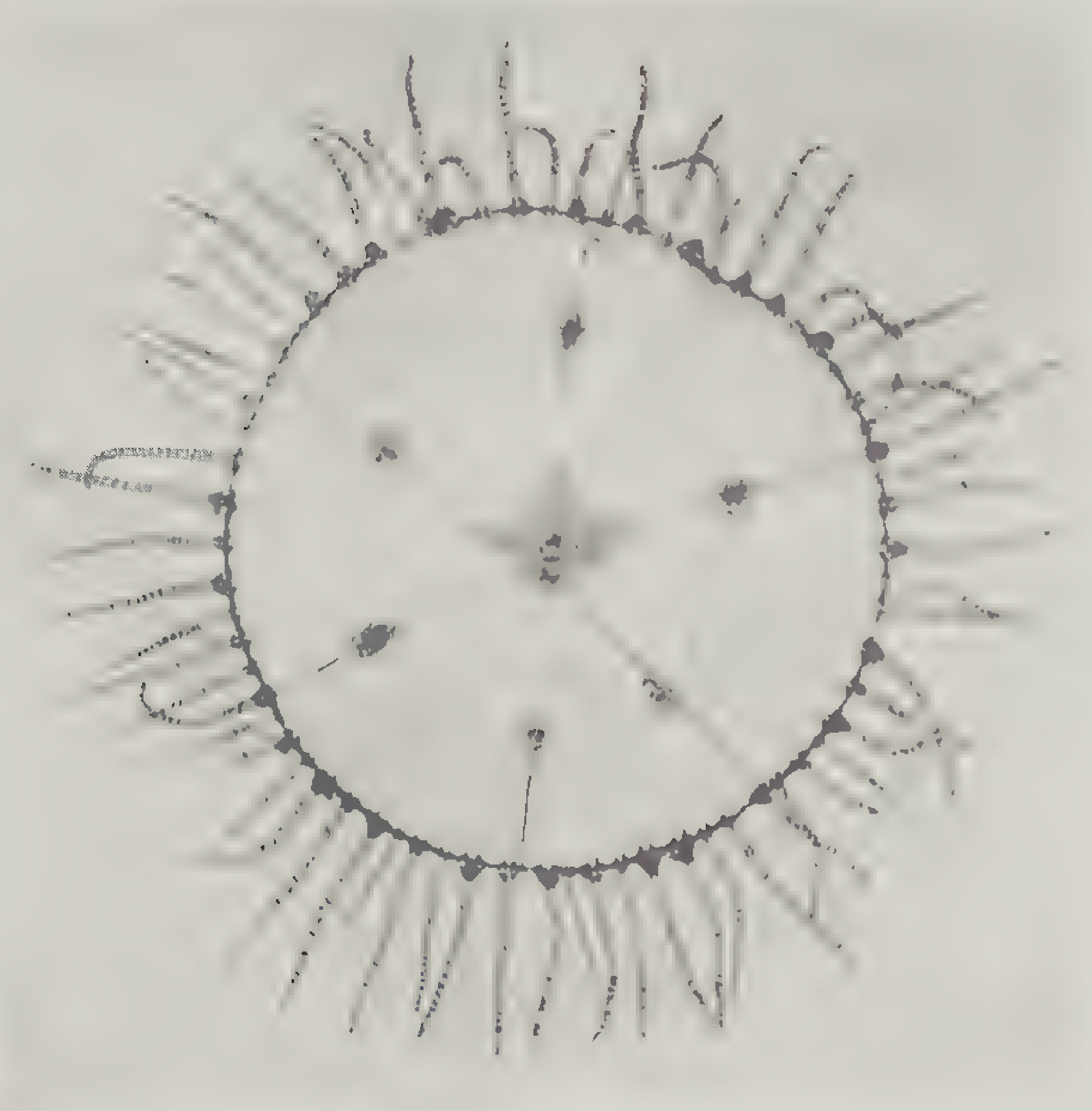

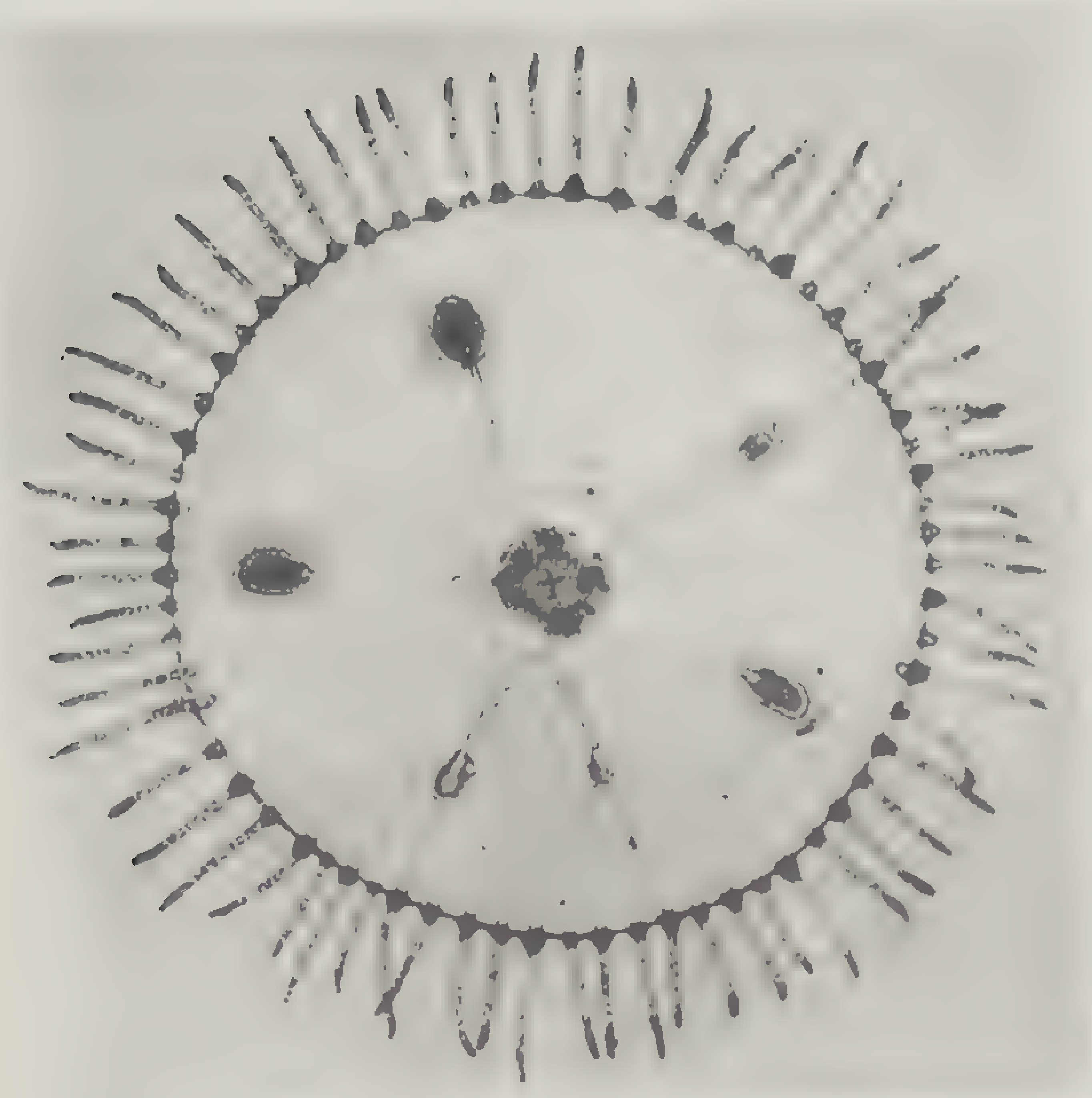

WOODWORTH PHOTO

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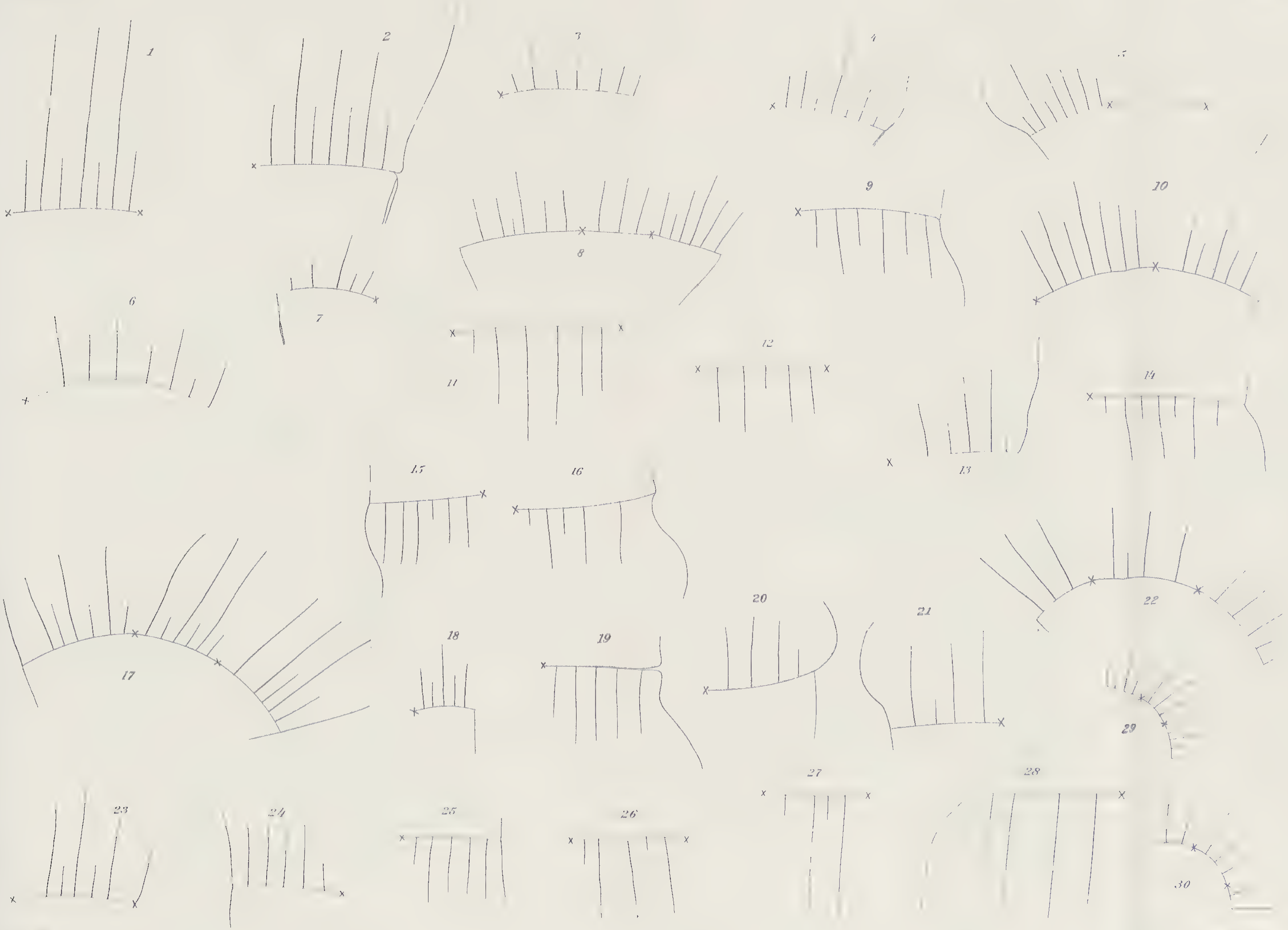
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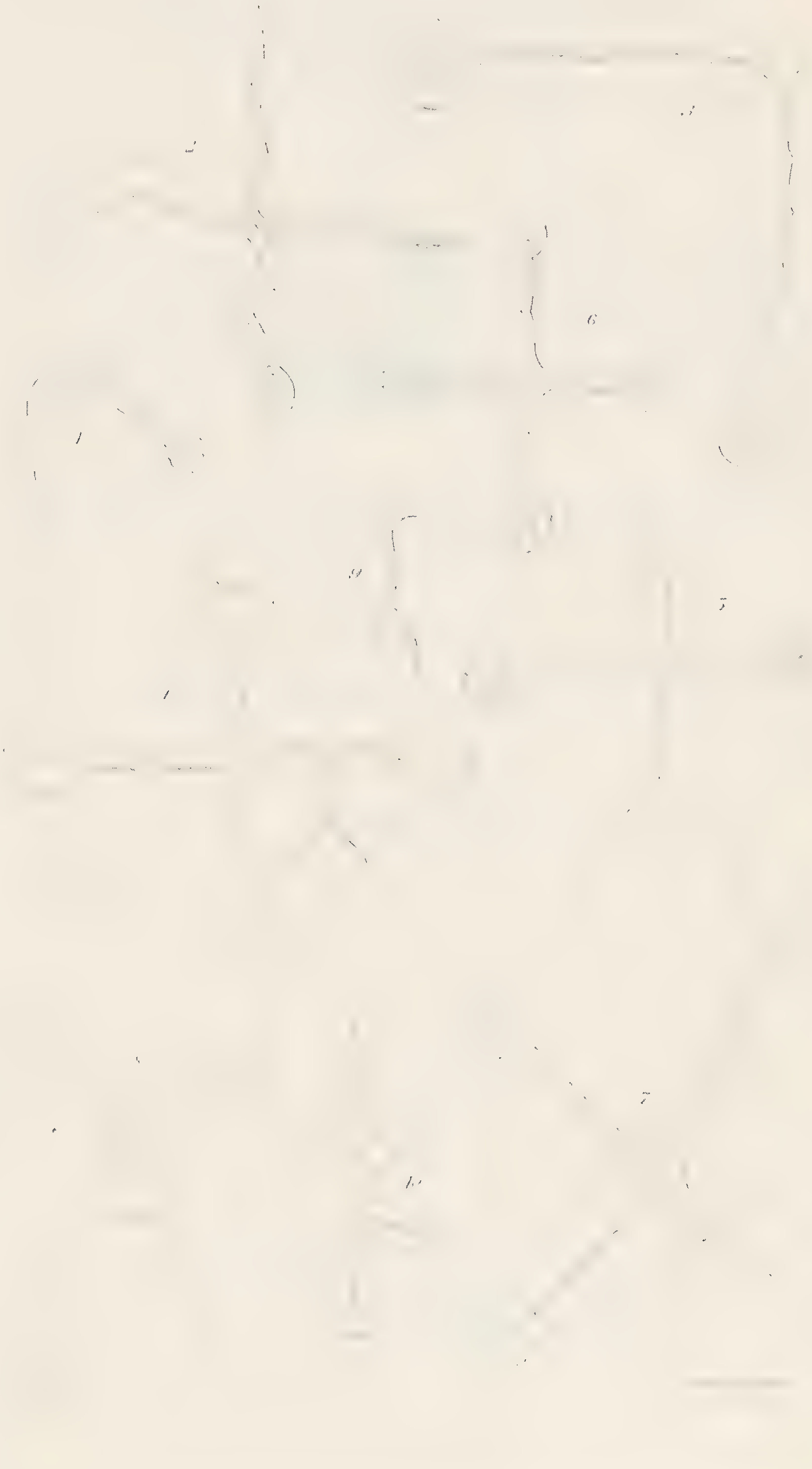

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Bulletin of the Museum of Comparative Zoölogy

AT HARVARD COLLEGE.

Vol. XXX. No. 3.

REPORTS ON THE RESULTS OF DREDGING, UNDER THE SUPERVISION OF ALEXANDER AGASSIZ, IN THE GULF OF MEXICO AND THE CARIBBEAN SEA, AND ON THE EAST COAST OF THE UNITED STATES, 1877 TO 1880, BY THE U. S. COAST SURVEY STEAMER "BLAKE," LIEUT.-COMMANDER C. D. SIGSBEE, U. S. N., AND COMMANDER J. R. BARTLETT, U. S. N., COMMANDING.

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XXXVII.

SUPPLEMENTARY NOTES ON THE CRUSTACEA.

By Walter Faxon.

With Two Plates.

CAMBRIDGE, MASS., U. S. A.:
PRINTED FOR THE MUSEUM.
November, 1896.

No. 3: — *Reports on the Results of Dredging, under the Supervision of* Alexander Agassiz, *in the Gulf of Mexico and the Caribbean Sea, and on the East Coast of the United States,* 1877 *to* 1880, *by the U. S. Coast Survey Steamer "Blake," Lieut.-Commander* C. D. Sigsbee, *U. S. N., and Commander* J. R. Bartlett, *U. S. N., Commanding.*

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XXXVII.

Supplementary Notes on the Crustacea. By Walter Faxon.

The following notes were made while identifying some of the "Blake" Crustacea that were retained as "duplicates" when the bulk of the collection was sent to A. Milne Edwards in Paris, and some (Macrura) that were returned by Milne Edwards undetermined. The notes chiefly consist of hitherto unpublished locality records, which add something to our knowledge of the distribution of many species. They also include descriptions of six new species (five Macrura and one Schizopod). Detailed lists of the dredging stations occupied by the "Blake" will be found in the Bulletin of the Museum of Comparative Zoölogy, Vol. VI. No. 1, and Vol. VIII. No. 4.

DECAPODA.

Anamathia hystrix (Stimps.).

Station 300. 82 fathoms. 1 ♂.

Anomalothir furcillatus (Stimps.).

Station 159. 196 fathoms. 1 ♀.
Off Port Royal, Jamaica. 100 fathoms. 1 ♀.

Pericera cornuta cælata (A. M. Edw.).

Station XX. 50 fathoms. 2 specimens.

Picroceroides tubularis Miers.

Station XXI. 33 fathoms. 1 ♂.

The rostral horns and preocular spines are longer than in the male specimen figured by Miers.

Lambrous pourtalesii Stimps.

Station XXX. 51 fathoms. 2 ♂.
" 133. 42 " 1 ♂.

Neptunus (Hellenus) spinicarpus (Stimps.).

Station 149. 60 to 150 fathoms. 1 ♀.

Achelous spinimanus (Latr.).

Station 144. 21 fathoms. 2 ♀.

Calappa flammea (Herbst).

Station 144. 21 fathoms. 1 ♂, 1 ♀.

Acanthocarpus alexandri Stimps.

Station 148. 208 fathoms. 1 ♂, 1 ♀.
" 149. 60 to 150 fathoms. 1 ♂.

Myropsis quinquespinosa Stimps.

Off Port Royal, Jamaica. 100 fathoms. 1 ♂.

Iliacantha subglobosa Stimps.

Station X. 103 fathoms. 1 ♀.

Cyclodorippe antennaria A. M. Edw.

Station 238. 127 fathoms. 1 ♀.
" 246. 154 " 2 ♀.
" 274. 209 " 1 ♂. 1 ♀.

Iconaxius caribbæus, sp. nov.

Plate I. Figs. 1-4.

Similar to *Iconaxius acutifrons* Bate, but different in the form of the rostrum, which is much broader than in *I. acutifrons*, less triangular in its outline, and broadly rounded at the anterior end; the upper border of the propodite of the larger cheliped, moreover, is entire, not denticulate as in *I. acutifrons*. The eyes are larger, and more heavily pigmented.

The margins of the rostrum are minutely denticulate, as in *I. acutifrons*, the median keel entire.

Length, 17 mm.

Station	166.	150 fathoms.	1 specimen.
"	232.	88 "	1 "
"	241.	163 "	3 "
"	283.	237 "	1 (type).

Lives as a commensal in Sponges of the genus *Farrea.*

The genus *Iconaxius*, of which four species have been previously described, has a wide distribution in the warm and temperate seas. It has been recorded from such remote localities as the Arabian Gulf, Banda Sea, Japan, Kermadec Islands, and the Gulf of Panama. It is now for the first time recorded from the Atlantic.

Polycheles crucifer (W.-S.).

Station	29.	955 fathoms.	3 specimens.
"	135.	450 "	1 "
"	179.	824 "	1 (exuviæ).
"	180.	982 "	1 specimen.
"	182.	1,131 "	1 "
"	188.	372 "	1 "
"	190.	542 "	1 "

Polycheles agassizii (A. M. Edw.).

Station	129.	314 fathoms.	3 specimens.
"	153.	303 "	1 "
"	238.	127 "	1 "
"	260.	291 "	1 "
"	XXVI.	297 "	1 "

Polycheles sculptus Smith.

Station	211.	357 fathoms.	3 specimens.
"	227.	573 "	1 "
"	230.	464 "	1 "

Station	245.	1,058 fathoms.	1 specimen.[1]
"	257.	553 "	2 "
"	265.	576 "	2 "
"	268.	955 "	1 "
"	VII.	610 "	1 "
"	XVIII.	600 "	2 "

Nephropsis agassizii A. M. Edw.

Nephropsis agassizii A. M. Edw., Ann. Sci. Nat., Zool., 6ᵉ sér., Vol. IX. No. 2, 1880.

Station	195.	502½ fathoms.	1 ♀.
"	200.	472 "	1 ♂.
"	227.	573 "	1 ♂.

Nephropsis aculeata Smith.

Nephropsis aculeata Smith, Proc. U. S. Nat. Mus., Vol. III. p. 431, 1881.

Nephropsis agassizii Smith, Bull. Mus. Comp. Zoöl., Vol. XV. p. 44, Fig. 240, 1888 (*nec* A. M. Edw.).

Nephropsis rosea (W.-Suhm MS.) Bate, Rep. "Challenger" Macrura, p. 178, Fig. 39, Pl. XXIII. Figs. 1, 2, Pl. XXIV. Fig. 1, 1888.

Station	185.	333 fathoms.	3 specimens.
"	188.	372 "	1 "
"	222.	422 "	3 "
"	226.	424 "	1 "
"	230.	464 "	1 "
"	?	? "	1 "

There are two species of *Nephropsis* in the West Indian region, *N. agassizii* A. M. Edw., with two pairs of lateral spines on the rostrum, and *N. aculeata* Smith, with only one pair of rostral spines. *N. agassizii* was very inadequately described by A. Milne Edwards, and the type specimen, from the Strait of Florida, 1,500 metres, has never been returned to Cambridge. Soon after, the other species, *N. aculeata*, was described by Smith from specimens obtained off the south coast of New England, in 100 to 126 fathoms. Subsequently Smith and other authors supposed that *N. aculeata* was identical with *N. agassizii*. The chief differences between the two species are the following. In *N. agassizii* the rostrum is armed with two or two and a half pairs[2] of lateral teeth; in *N. aculeata* there is only one pair of lateral rostral spines;

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[1] Identified as *P. agassizii* by A. Milne Edwards, and so recorded by him in Bull. Mus. Comp. Zoöl., Vol. VIII. p. 66, 1880.

[2] The third lateral spine may occur on either the right or the left side of the rostrum.

the shell is less coarsely granulated, but more spiny in the former species than in the latter; the two lines on the proximal half of the rostrum in both species, widely diverging as they pass backward over the gastric area, are marked by small tubercles in *N. aculeata*, by distinct acute spines in *N. agassizii;* the top of the small median tubercle on the gastric area is truncated in *N. aculeata*, while in *N. agassizii* it is bluntly triangular, passing into a slight median longitudinal carina both in front and behind; the abdominal pleuræ are produced into longer spines in *N. agassizii* than in *N. aculeata*, and the spines moreover trend more distinctly backward, forming a stronger angle with the vertical axis of the pleura; the outer surfaces of these pleuræ are quite smooth in *N. agassizii*, while in *N. aculeata* they are conspicuously granulated both on their margins and on the distinctly raised central field; the lateral borders of the abdominal terga, which form a festoon on each side of the abdomen, are more strongly convex in the former species; another distinction is apparent in the sixth abdominal somite, viz. in *N. aculeata* the antero-lateral margin of the pleura is shorter than the postero-lateral border, whereas in the other species the antero-lateral border is longer than the postero-lateral; the tergum of this somite in *N. aculeata* sends off a granulated ridge from near its posterior lateral angles, — a ridge which runs forward into the upper, depressed portion of the pleura; this ridge is not found in *N. agassizii.*

Nephropsis rosea Bate is without much doubt a young individual of *N. aculeata. N. atlantica* Norman[1] is very similar to *N. agassizii*, but has a sharp spine on the anterior margin of the second abdominal pleura.

Stenopus hispidus (Oliv.).

Station	11.	37 fathoms.	1 specimen.
"	12.	36 "	1 "
"	36.	84 "	1 "
"	132.	115 "	2 "

Pontophilus gracilis Smith.

Station	43.	339 fathoms.	1 specimen.
"	47.	321 "	24 "
"	48.	533 "	1 "
"	221.	423 "	1 "

Prionocrangon pectinata, sp. nov.

Plate II. Figs. 4-7.

Rostrum spiniform, inclined at an angle of 45° to the axis of the body. Median dorsal line of the carapace armed with a row of eight spiniform teeth, which extends backward nearly to the posterior border of the carapace. Anterolateral margins of the carapace angulated below the orbit. Telson much shorter than the appendages of the sixth abdominal somite, broad, with a pair of dorsal longitudinal ribs, abruptly contracted a short way beyond the middle; tip truncate, setiferous.

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[1] Proc Roy. Soc. Edinburgh for 1881-82, p. 684; Wood-Mason and Alcock, Ann. Mag. Nat. Hist., 6th Series, Vol. VII. p. 197, Fig. 4, 1891.

The eyes are absent; their peduncles are transformed into a pair of closely apposed trihedral processes, with acute and somewhat divergent tips. The first segment of the antennule is very long, reaching nearly to the end of the antennal scale; the second and third segments are, on the other hand, very short, the third bearing two flagella, the outer of which is very much shorter than the inner. The antennal scale is long and narrow, its outer margin lightly concave.

Length, 28 mm.

Station 201. Off Martinique. 565 fathoms. 1 ♀.

The rostrum is proportionally smaller than in *P. ommatosteres* Wood-Mason,[1] while the dorsal teeth of the carapace are larger, more numerous, and extend farther back on the cephalothorax; the telson is shorter; the antennal scale is longer than the proximal segment of the antennule. According to Wood-Mason, there is no trace of eyes or eye-stalks in *P. ommatosteres.* In *P. pectinata* there are distinct rudiments of the eye-stalks, as above described. *P. ommatosteres* comes from the Andaman Sea, 405 fathoms, and the Bay of Bengal, 200–350 fathoms.

Glyphocrangon aculeata A. M. Edw.

Station	29.	955	fathoms.	1	specimen.
"	163.	769	"	2	"
"	174.	878	"	1	"
"	190.	542	"	4	"
"	195.	502½	"	1	"
"	265.	576	"	2	"
"	VIII.	610	"	1	"

Glyphocrangon spinicauda A. M. Edw.

Station	148.	208	fathoms.	2	specimens.
"	274.	209	"	12	"
"	275.	218	"	6	"
"	281.	288	"	7	"

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[1] Ann. Mag. Nat. Hist., 6th ser., Vol. VIII. p. 362, 1891; Journ. Asiatic Soc. Bengal, Vol LXIII. p. 152, 1894; Ill. Zoöl. R. I. M. S. "Investigator," Crust., Pl. IX. Fig. 4, 1895.

Glyphocrangon nobilis A. M. Edw.[1]

Station	41.	860 fathoms.	6 specimens.
"	130.	451 "	2 "
"	162.	734 "	2 "
"	174.	878 "	4 "
"	176.	391 "	1 young.
"	179.	824 "	1 specimen.
"	185.	333 "	7 "
"	211.	357 "	1 young.
"	222.	422 "	2 "
"	227.	573 "	1 specimen.

Glyphocrangon neglecta, sp. nov.

Plate 1. Figs. 5, 6.

Rostrum longer than the rest of the carapace, trending a little downward for the anterior half of its length, then curving gently upward to the tip, which is slender and acute; the anterior half of the rostrum is distinctly carinated in the median line, but the carina fades away before attaining the base of the rostrum;

---

[1] The *Glyphocrangon* doubtfully referred to *G. nobilis* in my Report on the Stalk-eyed Crustacea of the "Albatross" Expedition of 1891 (Mem. Mus. Comp. Zoöl., Vol. XVIII. p. 142, 1895) is distinct from *G. nobilis*, as appears from an examination of a larger number of specimens of the latter species. In the "Albatross" species, which may be called *Glyphocrangon vicaria*, the upper surface of the rostrum is corrugated on each side of the median carina, in front of the anterior pair of lateral spines; in *G. nobilis* this corrugation does not exist. In *G. vicaria* the anterior moiety of the fourth or lateral crest of the carapace is broken into two parts by a deep notch; the part in front of the notch is produced anteriorly to form a strong spine, while the part behind the notch merely forms a projecting angle or shoulder; in *G. nobili* the anterior moiety of the fourth crest is continuous from the posterior end to the anterior spine. The tubercles of the first and second crests are more prominent and spiniform in *G. vicaria* than in *G. nobilis*. The dorsal carinæ of the telson are dentate anteriorly in *G. vicaria*, simple in *G. nobilis*. *G. vicaria* is even more closely related to *G. longirostris* Smith, which it represents on the Pacific side of the American continent. These are the chief differences between the two species: the rostrum, corrugated above in both species, is narrower in front of the anterior lateral spines in *G. vicaria* than in *G. longirostris*. The anterior moiety of the fourth lateral carina is broken into two distinct parts by a notch in the former, while it is merely sinuate in its outline in the latter. The tubercles on the first and second crests of the carapace are more prominent and spiny in the former than in the latter. The median dorsal crest of the abdomen, moreover, is more prominent. These differences, though very small, appear to be constant, and afford another instance of a slight divergence between two representative forms on the Atlantic and Pacific sides of the American continent. The type specimens of *G. vicaria* were dredged in 1189 fathoms, Lat. 0° 54′ N., Long. 91° 9′ W., "Albatross" Station 3411.

there are two pairs of lateral rostral spines, one of which lies in advance of the eyes, the other just behind the posterior wall of the orbit; on the lower face of the rostrum there appears just the slightest trace of a median longitudinal carina. The upper surface of the first or dorsal pair of carinæ is eroded; behind the cervical groove this pair of carinæ converge towards one another. Just in front of this pair of carinæ, lying in the median line at the base of the rostrum, is a small tubercle or papilla. In the interval between the first and second carinæ on each side are about four faint tubercles on the cardiac region, and on each side of the gastric region are four larger low tubercles, the hindmost of which is the largest of all. The anterior moiety of the third carina (adopting Wood-Mason's terminology) is well developed as a backward prolongation of the external orbital spine, which is long, acute, and inclined outward and upward. The fourth carina is also developed both anteriorly and posteriorly to the cervical groove, its anterior moiety being continuous with the antero-inferior, or branchiostegian, spine of the carapace. Barring the external orbital and branchiostegian spines, the anterior moieties of both the third and fourth carinæ are entire, without a trace of spine or tooth. The trend of the branchiostegian spine is nearly straight forward, its downward and outward deflection being very slight. With the exceptions noted above, the spaces between the carinæ of the carapace are pretty smooth.

The abdomen is lightly sculptured for the genus to which this species belongs. Only the first and sixth segments are conspicuously carinated above. The pleuræ of the second abdominal segment are one-toothed. The telson exceeds the last pair of abdominal appendages, and is rather abruptly bent upward at the tip.

Length, 75 mm.; cephalothorax including rostrum, 35 mm.; rostrum, 19 mm.; telson, 13 mm.

Station 261, off Grenada.	340 fathoms.	1 ♀ with eggs.	Type.
" 153, off Montserrat.	303	"	1 ♂.
" 260, off Grenada.	291	"	1 young.

This species is peculiar in having the anterior moiety of the third and fourth carinæ of the carapace well developed and continuous with the external orbital and branchiostegian spines respectively. In *G. gilesii* Wood-Mason, which also has the anterior portion of both the third and fourth crests developed, these crests are produced anteriorly into small spines independent of the external orbital and branchiostegian spines.

Stylodactylus serratus A. M. Edw.

Station 205.	334 fathoms.		3 specimens.
" 151.	356	"	1	"

Pantomus parvulus A. M. Edw.

Station 134	248 fathoms.	2 specimens.

Pandalus longipes A. M. Edw.

Station 274.	209 fathoms.	12+ specimens.
“ 291.	200 “	12+ “
“ 295.	180 “	2 “
“ 300.	82 “	12+ “

Pandalus ensis A. M. Edw.

Station 208.	213 fathoms.	1 specimen.
“ 258.	159 “	2 “

Pandalus leptocerus Smith.

Station 345.	71 fathoms.	1 specimen.

Heterocarpus lævis A. M. Edw.

Station XXVI.	297 fathoms.	1 specimen.

Heterocarpus alexandri A. M. Edw.

Station 196.	1030 fathoms.	1 specimen.

Heterocarpus ensifer A. M. Edw.

Station 146.	245 fathoms.	1 specimen.
“ 153.	303 “	1 “
“ 258.	159 “	2 “

Nematocarcinus cursor A. M. Edw.

Station 151.	356 fathoms.	12+ specimens.
“ 160.	393 “	2 “
“ 161.	583 “	1 “
“ 205.	334 “	2 “
“ 227.	573 “	2 “
“ 274.	209 “	1 “

Hoplophorus gracilirostris A. M. Edw.

Station 100.	250–400 fathoms.	1 specimen.
“ 191.	108–250 “	1 “
“ 226.	424 “	1 “
“ 230.	464 “	1 “
“ 258.	159 “	1 “
“ 271.	458 “	1 “

Acanthephyra affinis, sp. nov.

Plate II. Fig. 1-3.

Similar to *Acanthephyra* (*Systellaspis*) *lanceocaudata* Bate, but different in the following regards: the apical tooth of the antennal scale projects forward far beyond the membranous part of the organ; the telson is shorter than even the inner branches of the posterior pair of abdominal appendages, and its dorsal surface is flattened, but not grooved.

The seven teeth that surmount the gastric crest are closely approximated, and increase in size successively from the first to the fifth. The sixth is about equal to the fifth, the seventh a little smaller. The egg of this species measures 3 × 2 mm.

Length, 100 mm.

Station 258. 159 fathoms. 1 ♀.

This species belongs to the subgenus *Systellaspis*, in which the orbit is continuous to the first antennal tooth (the orbital tooth being absent), the dorsal carina of the sixth abdominal somite is wanting, and a prominent angle or tooth projects from each side of the anterior border of the first abdominal somite, overlapping the posterior margin of the carapace. The eggs, moreover, are of large size, indicating a protracted period of intra-oval development.

Acanthephyra debilis A. M. Edw.

Station 107. 428 fathoms. 1 specimen.

Acanthephyra armata A. M. Edw.

Station 135. 450 fathoms. 1 specimen.
" 151. 356 " 2 "

Sicyonia edwardsii Miers

Station 142. 27 fathoms. 1 specimen.

Sicyonia brevirostris Stimps.

Station 38. 20 fathoms. 1 specimen.

Peneus brasiliensis Latr.

Station 37. 35 fathoms. 2 specimens.
" 29. 955 " 3 young.

Parapeneus megalops Smith.

Station 147.	250 fathoms.	4 specimens.
" 148.	208 "	4 "
" 258.	159 "	6 "
" 275.	218 "	4 "
" 281.	288 "	10 "
" 283.	237 "	1 "

Parapeneus politus Smith.

Station 36. 84 fathoms. 27 specimens.

Haliporus debilis (Smith).

Station 47. 321 fathoms. 1 specimen.

Plesiopeneus armatus (Bate).

Station 31.	1,920 fathoms.	2 specimens.
" 187.	411 "	1 "

Hemipeneus triton Fax.

Station 227. 573 fathoms. 1 specimen.

Benthesicymus bartletti Smith.

Station 29.	955 fathoms.	1 specimen.
" 33.	1400–1568 "	1 "
" 163.	769–878 "	2 "
" 179.	824 "	1 "
" 190.	542 "	1 "
" 227.	573 "	2 "
" 245.	1058 "	1 "
" 265.	576 "	1 "
" 288.	399 "	2 "

Sergestes robustus Smith.

Station 205.	334 fathoms.	1 specimen.
" 211.	357 "	1 "
" 260.	291 "	1 "
" 264.	416 "	1 "
" 265.	576 "	2 "
" 267.	626 "	1 "

Sergestes mollis Smith.

Station 30. 968 fathoms. 2 specimens.

SCHIZOPODA.

Lophogaster longirostris, sp. nov.

Plate. II. Figs. 8-10.

Similar to *L. typicus* Sars, but different in the great length of the median spine of the rostrum, which far surpasses the antennular peduncle, and almost attains to the tips of the antennal scales. There are six teeth along the outer edge of the antennal scale. Length, 27 mm.

Station 50. 119 fathoms. 20 specimens.

Gnathophausia zoëa W.-Suhm.

Station	185.	333	fathoms.	2	specimens.
"	201.	565	"	1	"
"	221.	423	"	1	"
"	227.	573	"	1	"
"	228.	785	"	1	"
"	230.	464	"	1	"
"	284.	347	"	2	"
"	288.	399	"	3	"

Eucopia sculpticauda Fax.

Station 30. 968 fathoms. 1 specimen.

Petalophthalmus armiger W.-Suhm.

Station 29. 955 fathoms. 1 ♀

This is the specimen figured in my Report on the Stalk-eyed Crustacea of the "Albatross" Expedition of 1891, Pl. LIII. Fig. 2 (Mem. Mus. Comp. Zool., Vol. XVIII.).

STOMATOPODA.

Squilla empusa Say.

Station 36. 84 fathoms. 1 specimen (young).

Pseudosquilla ciliata (Fabr.).

Martinique. 1 specimen.

ISOPODA.

Bathynomus giganteus A. M. Edw.

Station 179. 824 fathoms. 1 specimen, 157 × 80 mm.
" VII. 610 " 1 " 107 × 49 "

According to Wood-Mason and Alcock (Ann. Mag. Nat. Hist., 6th Series, Vol. VII. p. 270, 1891), this remarkable Isopod was captured in the Bay of Bengal at a depth of 740 fathoms. Dr. Arnold Ortmann[1] has described a second species of *Bathynomus* (*B. dœderleini*), taken on the coast of Japan, near Enoshima, Sagarni Bay. The depth is not recorded.

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[1] Proc. Acad. Nat. Sci. Phila., 1894, p. 191.

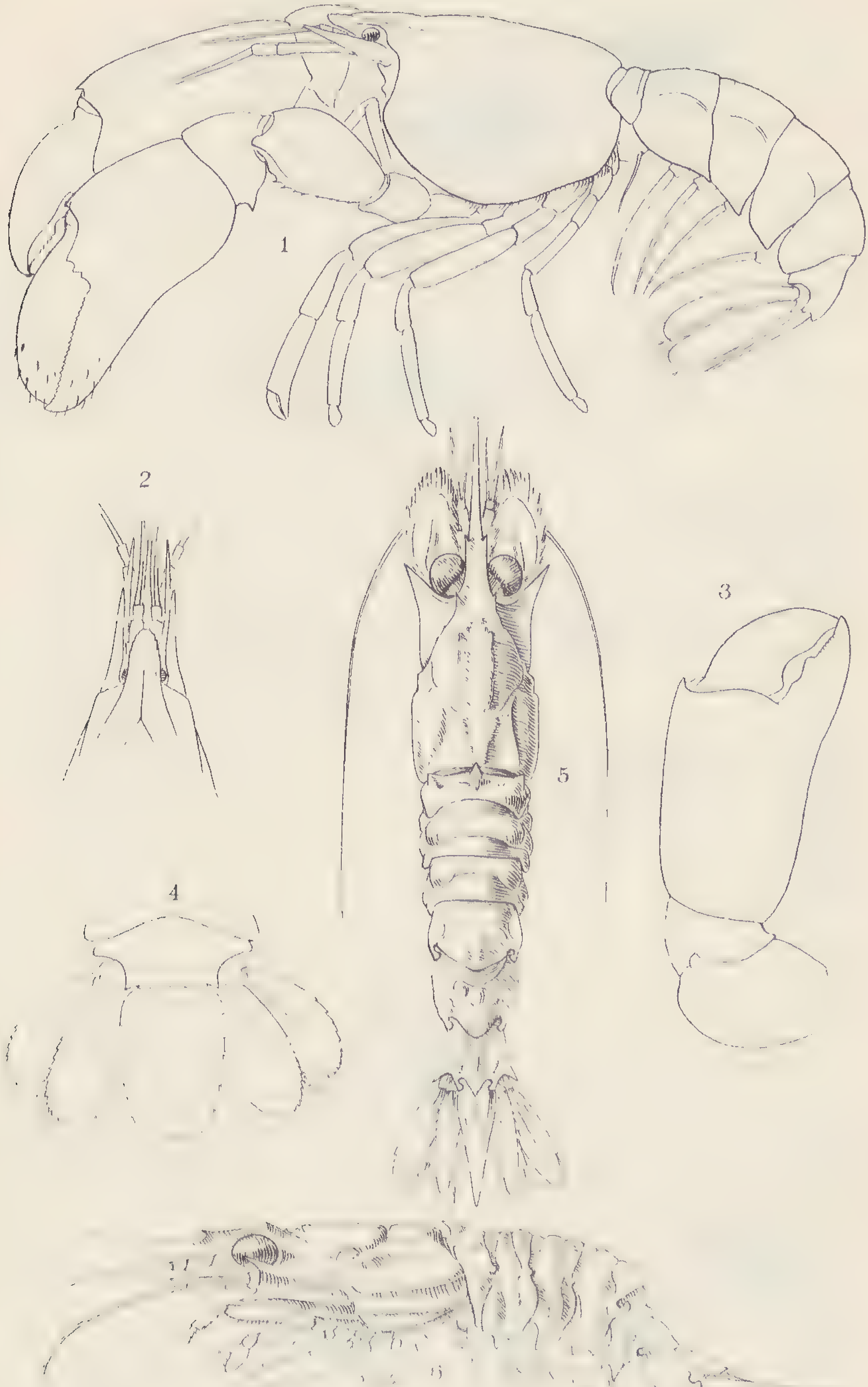
EXPLANATION OF THE PLATES.

PLATE I.

Fig. 1. *Iconaxius caribbæus* Fax. M. C. Z., No. 4195. Blake Sta. 283. × $5\frac{1}{3}$.
Fig. 2. The same. Head, from above. × $5\frac{1}{3}$.
Fig. 3. The same. Right chela, from the outside. × $5\frac{1}{3}$.
Fig. 4. *Iconaxius caribbæus* Fax. Telson and posterior pair of appendages. M. C. Z., No. 4147. Blake Sta. 241. Much enlarged.
Fig. 5. *Glyphocrangon neglecta* Fax. Female, dorsal view. M. C. Z., No. 4434. Blake Sta. 261. × $1\frac{1}{3}$.
Fig. 6. The same. Lateral view. × $1\frac{1}{3}$.

PLATE II.

Fig. 1. *Acanthephyra affinis* Fax. Female. M. C. Z., No. 4410. Blake Sta. 258. × $1\frac{1}{3}$.
Fig. 2. The same. Telson. × $1\frac{1}{3}$.
Fig. 3. The same. Antennal scale. × $1\frac{1}{3}$.
Fig. 4. *Prionocrangon pectinata* Fax. Female. M. C. Z., No. 4436. Blake Sta. 201. × 4.
Fig. 5. The same. Carapace, from above. × 4.
Fig. 6. The same. Chela. × 4.
Fig. 7. The same. Telson and posterior pair of abdominal appendages. × 4.
Fig. 8. *Lophogaster longirostris* Fax. M. C. Z., No. 4380. Blake Sta. 50. × 4.
Fig. 9. The same. Carapace, from above. × 4.
Fig. 10. The same. Telson and posterior pair of abdominal appendages. × 4.



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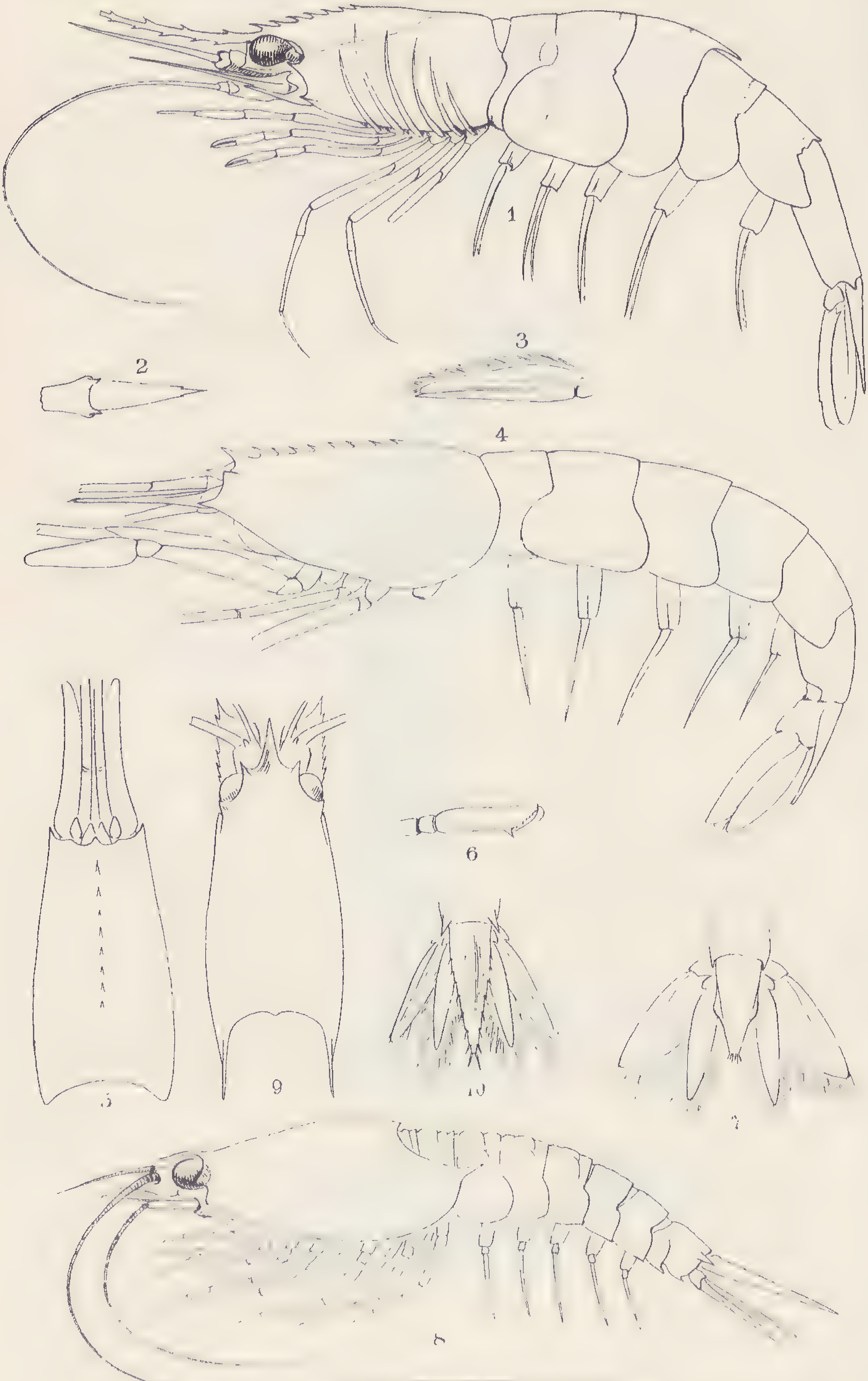

J. H. Emerton del.

Heliotype Printing Co.

1-3 Acanthephyra affinis Fax.
4-7 Prionocrangon pectinata Fax.
8-10 Lophogaster longirostris Fax.

Bulletin of the Museum of Comparative Zoölogy
AT HARVARD COLLEGE.
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ON THE COLOR AND COLOR-PATTERNS OF MOTHS AND BUTTERFLIES.

BY ALFRED GOLDSBOROUGH MAYER.

WITH TEN PLATES.

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No. 4.—*On the Color and Color-Patterns of Moths and Butterflies.*[1]

By Alfred Goldsborough Mayer.

This research is an investigation of the general phenomena of Color in Lepidoptera, and also a special account of the Color-Patterns of the Danaoid and Acraeoid Heliconidae, and of the Papilios of Tropical South America, and has been carried out under the direction of my friend and instructor, Dr. Charles B. Davenport; and the work was done in connection with one of the courses given by him in Harvard University in 1894–95.[2] I am indebted to Dr. Davenport not only for suggesting the subject, but also for his kindness in devoting much time to a criticism of the results.

The paper is divided into three parts. Part A contains an account of the general phenomena of color in Lepidoptera; Part B is devoted to a special discussion of the color-variations in the Heliconidae, with special reference to the phenomena of mimicry; and Part C consists of a summary of those results which are believed to be new to science. A Table of Contents is given at the end of the paper.

PART A.

GENERAL PHENOMENA OF COLOR IN LEPIDOPTERA.

I. Classification of Colors.

We follow Poulton ('90) in dividing Lepidopterous colors into (1) pigmental and (2) structural.

(1) *Pigmental Colors* are due to the presence of an actual pigment within the scales, and although such colors are very common in the Lepidoptera, it is frequently very difficult to say off-hand whether a given color is due to a pigment or to some structural effect. Coste ('90–'91) and Urech ('93) have, however, given criteria for determining whether a color is due to a pigment or to some other cause. They succeeded, for example, in dissolving out the color in many

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[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, E. L. Mark, Director, No. LXXIV.

[2] This paper was written in 1895 essentially as it now stands.

cases, leaving the wing white or colorless. Coste used as solvents a number of strong acids and alkalis; while Urech confined himself to the use of water, hydrochloric acid, and nitric acid. Their results may be conveniently summarized as follows:—

Black according to Urech is a pigmental color, for it may be dissolved out of the wings by means of hydrochloric or nitric acid.

Brown is usually insoluble in water, but is soluble in hydrochloric or nitric acid.

The *red and orange* pigments of the Pieridae, Lycaenidae, Nymphalidae, Zygaenidae, and some Papilios are soluble in water. They are insoluble in water in the Sphingidae, Arctidae, Bombycidae, Saturnidae, and Geometridae.

Yellow pigment is acted upon by reagents in almost the same way as the red and orange, especially if both red and yellow appear upon the same wing. It is soluble in the Pieridae, Lycaenidae, Nymphalidae, Satyridae, and some Papilios, but insoluble in the Sphingidae, Arctidae, Geometridae, and a few Noctuidae.

White is usually a structural color, but can be dissolved out from the wings of the Pieridae by water, being in this case, of course, due to a pigment.

Green pigment can be dissolved out by water in the cases of the Pieridae, Lycaenidae, and Geometridae. In the vast majority of cases, however, it is a structural color.

Violet and blue are almost always due to structural causes. In a few cases, however, as in Smerinthus ocellatus, a blue pigment can be dissolved out.

We see, then, that black, brown, red, orange, and yellow are usually due to pigment, while white, green, violet, and blue are generally due to structural effects.

It is well known that the scales of Lepidoptera are essentially hollow, flattened sacs often inclosing pigment, and Burmeister ('78) arrives at the conclusion, from a study of the scales in various species of Castnia, that the pigment is for the most part attached to the upper layer of the scale-sac, rendering it opaque, while the lower layer receives less pigment and is, in consequence, a little more translucent.

(2) *Structural Colors* owe their origin to the external structure of the scales or wing-membranes and not to the presence of a pigment. They are often caused by diffraction, due to the scales being covered with fine, parallel striae. Some of the most splendid colors in the

animal kingdom are due to this cause; such are the iridescent and opalescent hues of many of the Morphos and Indo-Asiatic Papilios. Very often the scales which display such brilliant colors contain no pigment whatsoever; for if one will merely soak them in alcohol, ether, or water, all color disappears, and the scales become as transparent as glass. This test was devised by Dimmock ('83), who used it upon the brilliantly colored scales of many beetles. It was first discovered by Burgess ('80), and has since been confirmed by Kellogg ('94), that the striae which produce these structural colors are all upon the outer surface of the scale, *i. e.*, the surface which is away from the wing-membrane and exposed to the light. Kellogg ('94) has determined the distance apart of the striae upon the scales of many species of Lepidoptera. It appears, for example, that the striae upon the scales of Danais plexippus are 2μ apart, those upon the transparent scales of Morpho sp. 1.5μ, upon the pigment-bearing scales of Morpho 0.72μ, and upon Callidryas eubule 0.9μ apart. It is very evident, then, that the brilliant coloration of the scales may be due to this fine striation, for the striae upon Rowland's or Rutherfurd's finest gratings are approximately 1.5μ apart, which is about the average distance between the ridges of the scales.

Structural colors are, however, not always due to diffraction; in the case of white, for example, the color is almost invariably due to a reflection of all, or nearly all, the light that impinges upon the scales. As long ago as 1855 Leydig pointed out that the silvery white color seen in the scales of some spiders, such as Salticus and Tegenaria, was due to air contained within them; and more recently Dimmock ('83) has shown that silvery white and milk-white colorations are due to optical effects produced by reflected light. In the silvery white scales, however, such as those of the under surface of the hind wings of Argynnis, there must be a polished reflecting surface toward the observer, for both silvery and milk-white colors appear simply milk-white by reflected light.

(3) *Combination Colors* owe their richness and brilliancy to a combination of structural and pigmental effects. The geranium-red spots upon the hind wings of the Mexican Papilio zeunis Lucas owe their red color to pigment, but over this red there plays, in certain lights, a beautiful pearly iridescence, which, in combination with the red, greatly enhances its charm. Urech ('92) has demonstrated that in the Vanessas there are scales which have chemical coloring matter

and interference colors also. In addition, he points out the interesting case of certain Lycaenidae where the scales exhibit to the eye only interference effects, and yet a pigment can be dissolved out of them by the use of water.

(4) *Quantitative Determination of Pigmental Colors.* I have analyzed the colors of many butterflies by means of the spectroscope, and also by Maxwell's discs. As is well known, Maxwell's discs are colored circular discs of cardboard, perforated at the center and slit along a radius so that two or more of them may be slid over each other, thus exposing different proportions of each. Then by rapidly rotating them the colors become blended, and thus it becomes possible to match any color, and to discover its fundamental constituents. By this means I have determined that the vast majority of the colors found in Lepidoptera are impure; that is to say, they contain a large percentage of black.

For example the white of the upper surface of the wings of the common Pieris rapae consists of: 17% black, 13% emerald-green, 10% lemon-yellow, and 60% white.

Also the so-called "blacks" found in butterflies are rarely jet-black, but, almost always, only deep shades of brown. For instance the deep brown color of the under surface of the wings of Heliconius melpomene consists of 93% black, 3% lemon-yellow, 3.5% of Maxwell's fundamental red (vermilion), and 0.5% of von Bezold's fundamental blue-violet.

The purest color I have met with is the canary-yellow ground color of the wings of Papilio turnus, which seems to consist of white light with the addition of a little yellow.

Other colors all possess considerable black. Thus the glaucous green of Colaenis dido consists of black 29%, vermilion 24%, emerald-green 37%, von Bezold's blue-violet 10%.

The sepia-brown ground color of Cercyonis alope consists of black 71%, vermilion 21.5%, emerald-green 7.5%.

The tawny rufous color of the wings of Mechanitis polymnia, etc., is made up of black 46%, vermilion 40%, lemon-yellow 14%.

The rufous red patch on the upper surface of the fore wings of Heliconius melpomene is made up of black 27%, vermilion 66.5%, lemon-yellow 6.5%.

The yellow of the fore wings of Mechanitis polymnia consists of lemon-yellow 67%, emerald-green 14%, and white 19%.

(5) *Spectrum Analysis of Colors of Lepidoptera.* I have made some spectrum analyses of the light reflected from the wings of various butterflies, by means of a piece of apparatus most kindly suggested for the purpose by Prof. Ogden N. Rood of Columbia College. The arrangement is shown in Figs. 1, 2, Plate **1**; Fig. 1 being a perspective view, and Fig. 2 a horizontal section of the apparatus, which consists of a rectangular box, blackened upon the inside, and having a well-fitting cover. A rectangular slit (O) was cut through one of the long sides of the box, near one end, and the other end of the same side was perforated in order to allow the admission of the direct-vision spectroscope (S). Imagine that we wish to examine the yellow spots from a butterfly's wing. All of the yellow spots from the wing are cut out, and pasted upon two pieces of cardboard so as to make two large unbroken patches of color. The pieces of cardboard are then blackened upon all those places where the colored wing was not pasted. One of the cardboards is then suitably mounted upon the back of the box at B; the other is placed upon a vertical support (F), the plane of which is parallel to the back of the box.

The working of the apparatus is as follows: the sunlight enters by the slit (O) and is reflected and diffused three or four times between the pieces of colored wing mounted upon the back (B) of the box, and the vertical support (F). The manner of this reflection and diffusion is shown by the dotted lines of Fig. 2. After undergoing several reflections, the light enters the direct-vision spectroscope (S). The slit of the spectroscope is wide open, and thus the light which enters it may readily be examined. It was found that it was necessary that the light be reflected more than once from the wing before it enters the spectroscope, for the first reflection shows so much white light that it is usually quite impossible to analyze the true color of the wing, the predominant colors being obscured by a continuous spectrum. In general it was found that the colors of the wings are not simple, but compound; that is to say, they are made up of a mixture of several different colors.

For example, the spectrum of the rufous ground color of the upper surface of the wings of Danais plexippus consists of all of the red and yellow of the spectrum and about 75% of the green.

The red spots upon the upper side of the fore wings of Heliconius melpomene also consist of the red and yellow and a very faint, hardly visible, trace of green.

The glaucous green patches on the wings of Colaenis dido are composed mainly of green and yellow, but there is also a faint development of about half of the blue and a still fainter trace of red.

The iridescent blue-green ground color of the upper surface of the wings of Morpho menelaus, viewed in such a way that the light makes an angle of about 20° with the normal to the surface of the wing, gives a spectrum of green and blue about equally developed.

The yellow ground color found on the upper side of the wings of Papilio turnus shows a continuous spectrum, in which the yellow seems to be rather more brilliant than in the normal spectrum of white light.

The sepia-brown ground color of the upper surface of the wings of Cercyonis alope gives a spectrum which lacks only the blue-green and blue.

(6) *Summary of Results.* The researches of Coste ('90–'91) and Urech ('93) have demonstrated that the colors of butterflies and moths may be produced by two causes: by the presence of an actual pigment, or by some structural effect. Some colors are due entirely to pigment, others to structural causes, and still others to a combination of the two.

Black, brown, red, orange, and yellow are invariably due to pigment.

Green is usually due to a structural effect, but in a few cases there is a green pigment present.

White, blue, and violet are almost invariably due to structural causes.

In addition to these facts I have found that most of the colors which are displayed by Lepidoptera contain a surprisingly large percentage of black. Also they are usually not simple colors, but composed of a mixture of several different colors. It is remarkable that Natural Selection, which is generally assumed to have been one of the principal factors in bringing about the wonderful development of colors in Lepidoptera, has not been potent enough to make these colors purer than is the case in existing butterflies.

II. The essential Nature of Pigmental Color in Lepidoptera.

(1) *Pigments of Larvae.* Poulton ('85) showed that the phytophagous larvae of Lepidoptera "owe their colour and markings to

two causes: (1) Pigments derived from their food-plants, chlorophyll and xanthophyll, and probably others; (2) pigments proper to the larvae, or larval tissues made use of because of some (merely incidental) aid which they lend to the colouring, *e. g.* fat." Poulton concludes that all green coloration is due to chlorophyll, and that nearly all yellows are due to xanthophyll. All other colors, including black and white and some yellows, are due to pigments proper to the larvae themselves.

Later, in 1893, Poulton proved that the larvae of Tryphaena pronuba could transform both etiolin and chlorophyll into a larval coloring matter, which may be either green or brown. It thus appears that some brown pigments are derived from food, and are merely modified plant pigments. Green larvae have green blood, and this color is due to chlorophyll in solution. It is remarkable that this chlorophyll solution is stable under the prolonged action of light, and in this respect is different from any other known solution of chlorophyll. It is worthy of note, further, that the spectrum of this green blood shows a great resemblance to that of chlorophyll. "In fact the two spectra are far nearer to one another than the ordinary spectrum of chlorophyll in alcoholic solution, is to the unaltered chlorophyll of leaves."

(2) *Pigments of Imagines.* In 1891, Urech showed that the similarity between the color of the urine of butterflies and the principal color of their scales is so close that it cannot be considered as accidental, but rather must be regarded as physiological. Urech compares in a table the color of the urine and that of the scales of 29 species of Lepidoptera. In all but two species the resemblance is very close.[1]

Urech further shows that the color of the urine (and the corresponding color of the scales) is not dependent upon the kind of food, for one and the same food plant may be differently digested in different groups of Lepidoptera. Thus he compares the behavior of a Vanessa with that of one of the Microlepidoptera (leaf-rollers). Both of these feed upon the nettle (Urtica). In the larva of the Vanessa the contents of the stomach are intensely green, but become red in the pupa. In the case of the leaf-roller the contents of the stomach are never markedly green and become insipid in color during the pupal stage.

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[1] Likewise, Hopkins ('94) has shown that in the Pieridae the urine is tinged by a yellow substance having exactly the color of the wings.

Poulton has shown that the reddish fluid voided by the Vanessas immediately after emergence from the chrysalis contains uric acid, and Hopkins ('94) says that when the yellow Pieridae emerge, they often void from the rectum a large quantity of uric acid. It should be borne in mind however, as Urech himself suggests, that the pigment found within the wings may not be identical in chemical composition with the similarly colored fluid from the alimentary tract.

Hopkins ('89, '91, '94, '96) has discovered that the white pigment found in the scales of Pieridae is uric acid, and that the red and yellow pigments of the Pieridae are due to derivatives of uric acid. He also says, "these uric acid derivatives used in ornamentation, are apparently confined to the Pieridae alone among butterflies." Hence when a Pierid mimics an insect of another family, the pigments in the two cases are chemically quite distinct. This is well seen in the genera Leptalis (Pieridae) and Mechanitis (Danaidae).

In addition to this, Griffiths ('92) finds that the green pigment found in Papilio, Parthenos, Hesperia, Limenitis, Larentia, Ino, and Halias is a derivative of uric acid, to which he gives the name of "Lepidopteric acid" and assigns the empyrical formula $C_{11} H_{10} Az_2 N_8 O_{10}$.

In a paper published in 1896 in the Bulletin of the Museum of Comparative Zoölogy at Harvard College, Vol. 29, I have shown, p. 226–230, that the pigments of the scales of Lepidoptera are derived by various chemical processes from the blood, or haemolymph, of the pupa, and that the haemolymph is a proteid substance containing egg-albumen, globulin, fibrin, xanthophyll, orthophosphoric acid, iron, potassium, and sodium.

III. Development of the various Colors in the Pupal Wings.

A few researches have been carried out upon this interesting topic, but as the literature is scattered and has never been brought together, it will perhaps not be amiss to present a brief résumé of the principal facts which have been already ascertained.

(1) *Historical Account of previous Researches.* In 1889 Schäffer ('89) discussed the question of the order and time of appearance of the colors in the pupal wings of several of the Vanessas. Unfortunately he apparently did not make his obser-

vations at sufficiently close intervals of time, and was, therefore, led into some misstatements, which have been corrected by van Bemmelen ('89) and Urech ('91).

Van Bemmelen carried out an elaborate research upon the development of the various spots and colors upon the wings of Pyrameis cardui, Vanessa urticae, V. io, Pieris brassicae, and a few other forms. He discusses in detail the time and manner of appearance of all of the different spots upon the wing. Into these details we shall not follow him, but shall merely present his general conclusions regarding the development of the various colors. In Pieris brassicae it appears that during the first days of the pupal stage the wings are colorless and transparent; after a few days, however, the fore wings become opaque, and white; later the hind wing, also, goes through the same changes. The wings then remain unaltered until about two days before the butterfly issues. Then, very suddenly, the black spots and the yellow ground tone of the under sides appear. White is thus the primary color; black and yellow secondary. The first color to make its appearance in the case of Pyrameis cardui is a brown-yellow ground color, which may be observed in pupae four days old. The hind wings are at this time somewhat darker than the fore wings. The color then changes from darker brown to cinnamon-brown. The black spots appear later upon this delicate reddish brown ground color. The three fused spots which form the whitish band in the middle of the front edge of the fore wing appear during the last days of development, just before the completion of the final color-pattern.

Both van Bemmelen and Urech have shown that in Vanessa urticae the order of appearance of the various colors is the same as in Pyrameis cardui. The first color to appear in Vanessa urticae is a faint reddish tinge; this deepens and forms the ground color, and later the black spots appear upon it.

Urech ('91) has made a careful study of the development of the colors upon the pupal wings of Vanessa io. The wings are at first wholly white. Then in a restricted area of this white is noticed the appearance of a yellow, which forms the yellow of the mature wings. Almost contemporaneous with the development of the yellow comes the red, which appears in another part of the primitively white field, and gradually deepens in color until it forms the brownish red ground color of the adult wings. Still later another portion of the primitive white changes into the black of the mature wing. The under side of the mature wings of Vanessa io is mainly uniform black, and in this case also this color develops from the white at a very rapid rate, near the end of the pupal stage. This development of the black directly upon the white areas is quite remarkable in Vanessa io, and very different from that of both Vanessa urticae and Pyrameis cardui, where the black spots develop upon a field already tinged with red. Urech points out the fact, that some of the white spots seen in the mature wings of the Vanessas represent the "primitive white" of the pupal wings.

Finally, the latest paper upon the subject of the development of color in the pupa is that of Haase ('93), who has examined the pupae of a number of Papilios (*e. g.*, philenor, machaon, asterias, turnus, and podalirius), and finds that during early pupal life the wings are as transparent as glass; after a time, however, they change to an impure white, which soon becomes yellowish, and then the various colors which are destined to adorn the mature wings begin to appear.

If we are to learn much of fundamental import concerning the phylogeny of color in Lepidoptera, the researches should be carried out upon the lower moths, and not upon such highly specialized forms of Rhopalocera as the Vanessae.

In my paper on Wing scales, etc. (Mayer, '96, p. 232), I have come to the conclusion that dull ocher-yellow and drabs are, phylogenetically speaking, the oldest pigmental colors in the Lepidoptera. The more brilliant colors, such as bright yellows, reds, and pigmental greens, are derived by complex chemical processes, and are, phylogenetically speaking, of recent appearance.

I have made a study of the development of the colors and pattern in the wings of Callosamia promethea Linn. and of Danais plexippus Fab.

(2) *Development of Color in the Pupal Wings of Callosamia promethea.* The cocoons of Callosamia promethea are very abundant during the winter months, when they may be found hanging to the stems of the food plants of the larvae. The pupal wings remain perfectly transparent all through the winter, until about ten days before the time when the moth is destined to issue; they then become opaque white. An examination of the wings at this period shows that the scales are perfectly formed (Fig. 25, Plate **3**), except for the

lack of pigment, which is developed later. If one treats the scales at this stage with oil of cedar-wood or clove oil, they become practically invisible under the microscope, thus demonstrating that there is no pigment within them. Fig. 26, Plate **3**, gives the appearance presented by a scale taken from the light drab-colored margin of the mature wing. This is about the lightest area upon the wing, except the white spots; but it will be seen that this scale is much darker in appearance than the unpigmented one shown in Fig. 25. The white or unpigmented condition of the wing lasts for about four days. The wings then become uniformly tinged with an impure yellow or light drab, and very soon after this the colors begin to make their appearance. They first appear upon the lower surface of the wings. Fig. 28, Plate **3**, represents the under surface of the fore wing of a female in a very early stage of color development; in fact the upper surface shows, as yet, no trace of the colors. It will be seen that a few dark red streaks have appeared near the central portion of the wing, and it is worthy of note that these occupy the *interspaces between the nervures.* The ocellus near the apex of the wing appears faintly outlined upon its background of impure yellow.

Fig. 27, Plate **3**, represents the under side of a hind wing of a male in about the same stage as Fig. 28. Here, again, the red color occupies the *interspaces*, and indeed it is only later that the nervures become clouded over by it.

Figs. 29 and 30, Plate **3**, represent, respectively, the under and upper sides of the fore wing of a male about five hours after the first appearance of the colors. Upon the upper side (Fig. 30) we see two gray streaks near the base of the wing and a light cinnamon-brown color extending from the lower edge toward the middle of the wing. The ocellus near the apex is now quite apparent, but still faint in color. On the under surface (Fig. 29) the red markings have developed to a much greater extent than in Fig. 28. The outermost of the two white spots which occupy the center of this red area becomes the white central spot of the mature wing; the innermost one is soon obliterated owing to its becoming clouded over with red.

Figs. 37 and 36 represent respectively the upper surface of the fore wing and the lower surface of the hind wing of a female, slightly more advanced than in Fig. 30. Fig. 31 represents a male and Fig. 38 a female about twelve hours after the first appearance of the color. It is remarkable that in this stage the male and female wings are quite similar in general appearance, except that the ground color of the male is now a dusky gray, while that of the female is a cinnamon-brown.

From this time onward, however, the wings of the two sexes begin to differ more and more in appearance, for the ground color of the male becomes deep black, while that of the female remains cinnamon-brown. This change is well exhibited by Figs. 32 and 39, Plate **3**, which give the appearance of the upper surfaces of the male and female wings respectively at about twenty hours after the first appearance of the colors. Fig. 33 represents the hind wing of the same male whose fore wing is shown in Fig. 32. Figs. 34, 35, 40, and 41 give the appearance of the pupal wings just before emergence, when the colors are completely formed.

To summarize; Figs. 27, 29, 33, and 35 give successive stages in the development of color in the male; and Figs. 28, 36–41 give similar stages for the female. It becomes evident, from a comparison of these successive developmental stages, that the colors appear first upon the central portions of the wings, and that the outer and costal edges of the wings and the nervures are the last parts to acquire the mature coloration.

It is worthy of remark that the color-pattern of the mature male Callosamia promethea is quite a departure from the type of coloration which is commonly found among the Saturnidae. The *female*, however, conforms very well to the general pattern of the other species of the family. It is quite evident that the deep black coloration of the male is, phylogenetically speaking, a new acquisition, and that the coloration of the female represents the less differentiated and therefore, more primitive type.

It is interesting in connection with these facts to observe that the color-patterns of both male and female develop in almost identical ways up to the twelfth hour after the first appearance of the color; that then, however, the grayish ground color of the male wings begins to deepen into the characteristic jet black of the adult, while the light cinnamon ground color of the female merely becomes slightly darker as the wings mature.

(3) *Development of Color in the Pupal Wings of Danais plexippus.* Figs. 42–45, Plate **3**, are intended to illustrate four stages in the development of color in the pupal fore wings of Danais plexippus. The pupal stage of this species is of brief duration, last-

ing from one to two weeks only, according to the temperature to which the chrysalis is exposed. For the first few days the wings are perfectly transparent, but about five days before the butterfly issues they become pure white. An examination of the scales at this period shows that they are completely formed and merely lack pigment. In about 48 hours after this (see Fig. 42) the ground color of the wings changes to a dirty yellow. It is interesting to note that the white spots which adorn the mature wings remain pure white. Fig. 43 illustrates the next stage, where the black has begun to appear in the region beyond the cell. The nervures themselves, however, remain white. Fig. 44 shows a still later condition, where the dirty yellow ground color has deepened into rufous, and the black has deepened and increased in area and has also begun to appear along the edges of the nervures. In Fig. 45 the black has finally suffused the nervures, the base of the wing and the submedian nervure being the only parts that still remain dull yellow. It is apparent that in Danais plexippus, as in Callosamia promethea, the central areas of the wings are the first to exhibit the mature colors, and that the nervures and costal edges of the wings are the last to be suffused.

IV. The Laws which govern the Color-Patterns of Butterflies and Moths.

(1) *Historical Account of previous Researches.* The earliest paper upon this subject is by Higgins ('68). He came to the conclusion, that "the simplest type of color presents itself in the plain uniform tint exhibited when the scales are all exactly alike." He also thought it probable that "the scales growing on the membrane upon or near the veins would be distinguished from the scales growing on other parts of the membrane by a freer development of pigmentary matter, and that in this manner would arise a kind of primary or fundamental color-pattern, namely, a pale ground with darker linear markings following the course of the veins, *e. g.* Pieris crataegi." He also attempted to explain the formation of eye-spots by assuming that crescent-shaped markings migrate outwards from the sides of the nervures and meet so as to inclose a space.

It is, however, untrue that there is a freer development of pigment within the scales lying upon the nervures; in fact, the reverse is the case, as we have seen, in both Danais plexippus and Callosamia promethea. Higgins's explanation of the formation of eye-spots is also fallacious.

Darwin ('71, Vol. 2, p. 133) published four excellent figures from a drawing by Trimen, illustrating two simple ways in which *eye-spots* are actually formed, both diametrically opposed to Higgins's hypothesis. Darwin says that in the South African butterfly, Cyllo leda, "in some specimens, large spaces on the upper surface of the wings are coloured black, and include irregular white marks, and from this state a complete gradation can be traced into a tolerably perfect ocellus, and this results from the contraction of the irregular blotches of colour. In another series of specimens a gradation can be followed from excessively minute white dots, surrounded by a scarcely visible black line, into perfectly symmetrical and large ocelli" with several rings.

Scudder ('88–'89) and, afterwards, Bateson ('94) have shown that the ordinary eye-spots, such as those found in Morpho and the Satyridae, are invariably placed in the interspaces between the longitudinal veins of the wings, and also that they are often found repeated upon homologous places of both pairs of wings. Bateson says that ocelli are often seen upon both surfaces of the wing, the centers of the upper and lower ocelli coinciding. In the majority of cases, however, the upper and lower ocelli, although coincident, have quite different colors. The simpler sort of ocelli, such as those seen in the Satyridae or in Morpho, have their centers on the line of the fold-marks or creases of the wing. It sometimes happens that these creases seem to begin from the center of an ocellus. As these creases commonly run midway between two nervures, it usually results that the center of the eye-spot is exactly half way between two nervures. The large eye-spots of Parnassius apollo are an exception to this rule. In some Morphos, Satyridae, etc., in cell I^b of the hind wing there are often two creases and two eye-spots, one for each crease; but if there be only one eye-spot present, its center does not correspond with the middle of the cell, "but is exactly upon the anterior of the two creases." I have observed the same law for the white marginal spots in cell I^c in Ceratinia vallonia, C. fimbria, and Mechanitis polymnia.

In 1889 Scudder, in his work upon the Butterflies of New

England, called attention to the following facts: the transverse series of dark spots so often seen in the body of the wings of Lepidoptera are invariably placed *in the interspaces between* the longitudinal veins, never upon the veins themselves, excepting only in rare instances, where the spots occur at the extreme margin. He also pointed out that in many types of moths all differentiation in coloring has been greatly retarded, so far as the hind wings are concerned, by their almost universal concealment by day beneath the overlapping front wings. In these cases "the simplest departure from uniformity consists of a deepening of the tint next the outer margin of the wing." It is but a step from this condition to a band of dark color or a row of spots parallel with the margin. This explains why the transverse style of markings, for the hind wings at least, is so common. Scudder showed that "the number of instances, in butterflies, in which similar markings appear in the same areas of the two wings, and in the same relative position in these areas, is far too common to be a mere coincidence. It is most readily traced in the disposition of the ocelli, which are very apt to be similar in size and perfection, and to be situated between the same branches of homologous veins."

(2) *Laws of Color-Patterns.* As a result of my own study of the wings of moths and butterflies, I am prepared to propose the following additional laws of color-patterns. (*a*) Any spot found upon the wings of a moth or butterfly *tends to be bilaterally symmetrical both as regards form and color*, the axis of symmetry being a line passing through the center of the interspace in which the spot is found, and parallel to the direction of the longitudinal nervures. For example, in Figs. 6 and 7, Plate **2**, each spot is bilaterally symmetrical about the axis HH. The same law holds for the spots represented in Figs. 8–14 and 16.

(*b*) Spots tend to appear *not in one interspace only*, but as a row occupying homologous places in successive interspaces. Indeed we almost always find similar spots arranged in linear series, each similar in shape and color to the others and occupying the center of its interspace. The rows of spots represented in Figs. 8–14 and 16 will suffice to illustrate this law.

It is interesting to notice that bands of color are often made by the fusion of a row of adjacent spots; and, conversely, chains of spots are often formed by the breaking up of bands, leaving a row of spots occupying the interspaces. Many instances of this are to be seen in certain specimens of various species of the Heliconidae. For example, in Heliconius eucrate (Fig. 58, Plate **4**) I have observed that certain specimens show a row of distinct spots in place of the, usually entire, band which crosses the middle of the hind wing. In fact, the vast majority of bands can be analyzed into a series of similar elements, each element occupying an interspace. Thus, in Plate **2**, Fig. 17, which represents a wing of Saturnia spini, the band seen crossing the wing parallel with its margin is made up of a series of fused crescents, each crescent occupying an interspace.

If, on the other hand, this band were to break away from the nervures, the result would be a series of crescent-shaped spots each occupying the center of an interspace. It is very interesting to observe the manner in which bands degenerate and disappear. Numerous opportunities for doing this may be had among the Heliconidae. In some species, as in Melinaea parallelis, hardly any two specimens are alike in the condition of the black band across the middle of the hind wings. *The most common method of disappearance is a shrinking away of the band at one end.* This is well illustrated in Figs. 84–87, Plate **7**, which represent a sort of "Mercator's Projection" of the wings of Mechanitis isthmia (for explanation of the plan of projection see page 207.) Fig. 84 represents a male, showing a well-marked band of hardly separated spots extending across the middle of the hind wing. Fig. 87 shows a female in which the spots are thinner and more crescentic and the separations much more marked. Fig. 85 is also drawn from a female, in which it will be seen that the band has shrunk away leaving only a portion of it at the right, and in Fig. 86, which represents another female specimen, only one faint spot is left.

It is very common to find bands shrinking away at *one* end. Sometimes, however, they shrink away at *both* ends, and very often they break up into a row of spots, which may then contract into the centers of their interspaces and finally disappear. It is worthy of note that it is *very* rare to find a band breaking at the middle of its length and each half receding from the other. Such a case is, however, shown by Melinaea parallelis (see Fig. 82, Plate **7**), where one sometimes finds specimens in which the black band across the middle of the hind wings is complete and unbroken; whereas in other specimens, as in Fig. 82, it is partially broken in the middle, and in still others the break has become a wide gap by the drawing away of the halves of the band from each other.

We see, then, that it is very common to find bands shrinking away from either end, but very rare to find them broken in the middle region. This, however, is only a special case of the law enunciated by Bateson ('94), that the ends of a linera series are more variable than the middle. Almost any row of spots also exhibits the same law, in that the spots occupying the middle portions of the row are similar one to another, while those at the ends of the series depart more or less from the type. (See Figs. 10–13, Plate **2**.)

The position of spots which are situated near the edge of the wing is largely controlled by the wing-folds or creases. In Melinaea egina (Fig. 96, Plate **8**) there is a row of white spots near the outer edges of the wings, and each of these spots is cut in two by a narrow black line which extends along the wing-fold. Also in Ceratinia vallonia (Fig. 81, Plate **7**) and in many other forms of the Danaoid Heliconidae one often finds two creases in a cell, and in this case there are two marginal spots, one on each crease. In many other cases, however, the marginal spots are double in each cell, although there is but a single wing-fold; the spots in these cases are situated at some distance on either side of the fold. (See Figs. 95, 96, Plate **8**.) Another very common condition is exemplified in Fig. 83, Plate **7**, where there is a single marginal spot situated upon the wing-fold in each cell.

(3) *Detailed Discussion of the Laws of Color-Patterns.* Figs. 6–14 and 16, Plate **2**, are taken from special cases which serve to illustrate the two chief laws of color-pattern, *i. e.*, that spots tend to be bilaterally symmetrical about an axis (HH, Figs. 6, 7) passing through the center of the cell parallel with the nervures; and also, that spots of similar shape and color tend to be repeated in a row of adjacent cells.

In Fig. 7 the spots are separated in the middle, but still incline outward symmetrically from the center; indeed, instances of double spots are very common. In such cases, however, each half spot is a reflection of its mate on the other side of the axis passing through the center of the cell.

Fig. 8 represents various eye-spots found in the Morphos, and will serve to illustrate the laws of eye-spots which have been enunciated by Scudder ('89) and Bateson ('94). These spots occupy the center of the cells in which they are found. In cell II, for example, is a large eye-spot with a crescent in its center, and it will be observed that this crescent follows the general law and is bilaterally symmetrical about the usual axis.[1]

Fig. 9 shows the law of repetition of some very complex spots, each being bilaterally symmetrical. It is found in Parthenos gambrisius.

Figs. 10 and 11 represent Ornithoptera urvilliana and O. priamus respectively. In Fig. 10 we see an instance of a spot within a spot, and in Fig. 11 an even more complex case, for here there are three systems of spots one within another.

Fig. 12 represents the marginal markings found in Hestia jasonia and Fig. 13 Hestia leuconoe var. clara. These two examples are intended to illustrate the fact, that, although the markings are situated *upon* the nervures, they are bilaterally symmetrical not *about the nervures* as axes, but about the usual axis passing midway between the nervures. In Fig. 12 it will be seen that the two curved markings situated upon nervures 1^b and 2, and projecting into cell I^c, are bilaterally symmetrical only in reference to the axis through the middle of the cell.

In allied species the spot situated upon nervure 1^b is often absent. The system of markings is therefore undergoing degeneration at this end (cf. Fig. 13, cell I^c). The curved mark upon nervure 5 (Fig. 12) projecting into cell V is plainly symmetrical with respect to its fellow in the opposite side of cell V, and not with its near companion which projects into cell IV. The same is also true in the case of the spots in cell VI.

In Fig. 13 the spots appear at the first glance to be bilaterally symmetrical about both nervures and centers of cells, but in cell IV the marking situated on nervure 4 does not quite reach to the center, and it is interesting to observe that its fellow on nervure 5 also falls short of reaching the center and is therefore symmetrical with respect to the other curved spot in cell IV. This case also furnishes an instance of a break in the middle of a linear series.

Fig. 14 is taken from the under surface of the hind wing of Papilio emalthion. It serves to illustrate the fusion of two originally separate rows of spots. In this case the crescent-shaped spots above have fused with the rectangular ones below, so as to inclose a portion of the ground color of the wing. Sometimes two rows of spots of different colors fuse, giving a chain of spots which are of one color above and another below.

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[1] A very beautiful exception (Fig. 19, Plate 2) to this rule for the crescents found in eye-spots is seen in the under surface of the fore wing of Missanga patinia Moore. It will be noticed that the large black crescent found in this beautiful eye-spot is 90° away from its usual position. This is the only exception of the sort known to me.

In Fig. 16 the spots composing the row BB are blue (dark) above, and red (light) below. It will be observed that the color is bilaterally symmetrical, as usual, about the axis through the middle of the cell. Such bicolored spots are often due to a simple fusion, as before stated; but sometimes they may, perhaps, be intrinsically bicolored.

Fig. 15 is a beautiful instance of an exception to the general rule that spots are bilateral about the axis through the center of the cell. It is taken from Ornithoptera trojana Staudinger.[1] The light spots represented near the outer edge of the wing are of a brilliant iridescent green. It is evident that they are distinctly bilateral with respect to the *nervures;* especially is this true of the pair adjacent to nervure 1 . Ornithoptera brookiana Wallace illustrates another exception, though in a less marked degree.[2] Other allied species of Ornithoptera, however, would seem to show that these apparent exceptions may have been derived from forms which exhibited two spots in each cell and followed the usual rule. These are the only instances of such exceptions known to me. I do not doubt, however, that further study would reveal others.

In Fig. 17 an example is given of the peculiar kind of eye-spots found in the Saturnidae. The species from which the figure was taken is Saturnia spini. It will be seen that this so-called eye-spot is quite different in formation from the ocelli of butterflies. It is simply a series of curved cross-bands between nervures, arranged symmetrically on both sides of the cross vein CC. The "eye-spots" upon the wings of Attacus luna and in the genus Telea are also of this sort. True eye-spots, however, similar to those found among the Morphos and Satyridae, occur in moths, as in the apex of the fore wing of Samia cecropia, Callosamia promethea, etc. "False" eye-spots are also found on the wings of butterflies; in Vanessa io, for example, the so-called eye-spot of the fore wing has been shown by Dixey ('90) to be made up of a series of fused spots. It will be remembered that Merrifield ('94, Plate 9, Fig. 4) caused this "ocellus" to break up into its constituents by subjecting the pupa to a temperature of 1° C. The ocellus upon the hind wing of Vanessa io is no doubt a true eye-spot; the only evidence which

[1] See Watkins, '91, Plate 4

[2] See Hewitson, '50-'76, Vol. 1.

might lead one to infer that the ocellus of the fore wing was of the same character is, that an aberrant form is sometimes found in nature having the "eye-spots" on both fore and hind wings obliterated, thus indicating a *possible* connection between the two (see South, '89).

Fig. 18 is intended to illustrate the process of degeneration occurring in bands. Band BB is represented as breaking down by the rare method of parting in the middle. Example, Melinaea parallelis. Band EE is degenerating at one end; this is a very common method.

Figs. 20–23 represent hypothetical conditions not found in nature; all being contrary to the conditions of the laws which have just been stated.

In Fig. 20 row RR presents three spots for each cell. I believe this has not been found in nature, but I should not be surprised if it were discovered, for it is not contrary to any of the laws.

Row CC, on the other hand, is contrary to the law of bilaterality, the crescents not being bilateral about axes passing through the middle of the interspaces parallel with the longitudinal nervures.

Fig. 21 is intended to show a series of spots arranged side by side in twos in each cell, and of different colors. This, I believe, is impossible, for it is contrary to the law of bilaterality of color arrangement about the usual axis (HH, Figs. 6, 7).

In Fig. 22 there are several conditions which are impossible; *e. g.*, an eye-spot situated upon a nervure is never seen in nature, also two spots originally side by side, as in cell III, *never rotate* around each other so as to come to lie one above the other. Spots often move, however, as shown by the arrows in cell IV, thus giving rise to fusions; or they may move away from each other, causing a wider gap between the rows. In cell I^b are shown two looped spots. One form (A) is quite usual, being found indeed in Cymothoe caenis Drury.[1] The other form of spot (D) is an impossibility, not being bilaterally symmetrical.

Fig. 23 illustrates other impossibilities in color-pattern, none of them, of course, being found in nature. For example, one never finds a row of slanting spots such as SS. Also one never sees a row of similar spots in *alternate* interspaces, such as is shown in DD, for this would be contrary to the law that similar spots are repeated in a row of adjacent interspaces. These last four diagrams

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[1] See Cramer (1779–'82), Vol. 2, Plate 146.

(Figs. 20–23) have been introduced merely to give an idea of the curiously strict limitations which nature has imposed upon the differentiation of the color-pattern. Many beautiful effects might have been produced, such for example as that of alternate interspaces showing different colors, but this is not seen in nature.

It is interesting to recall the fact, that the colors themselves are impure and by no means so brilliant as they, perhaps, might have been, had Natural Selection been more severe in regard to color.

There is doubtless some physiological reason why spots almost invariably appear and disappear in the *middle of the interspaces*, and when we know more of the anatomical and histological conditions of the wing during the development of the colors, we may be able to discover it. It will be remembered that in the developing pupal wings of Callosamia promethea and Danais plexippus I found that the colors first made their appearance in the interspaces, and finally spread out so as to tinge over the nervures.

(4) *Origin of Color-Variations.* There is every reason to believe that all kinds of spots and bands, which are essentially only fused spots, may appear or disappear in any individual specimen without going through a long course of Natural Selection and slow phylogenetic differentiation. Darwin and Trimen ('71) and Bateson ('94) have demonstrated that this is true for eye-spots. In the Heliconidae I have found that bands and rows of spots are very variable in different specimens of the same species (see Plate **7**, Figs. 84–87).

There is a large and widely scattered literature recording the appearance and disappearance of colors and markings upon the wings of Lepidoptera. Limits of time and space prohibit my doing justice to it here, but it may be well to call attention to a very few of the more recent papers upon the subject. Many of the color-aberrations recorded in this list of papers may be due to the direct influence of environmental conditions upon the individual, but others are no doubt true sports or, to speak crudely, "congenital" variations, and might under favorable conditions of life become the ancestors of *new* varieties or species. It seems highly probable that new species often arise from just such sports in the manner so frequently and ably expounded by Bateson.

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(5) *Climate and Melanism.* Lord Walsingham ('85), in his presidential address before the Yorkshire Naturalists' Union, brought forward the idea, that, although Arctic insects might be perfectly

able to withstand the most severe cold while in hibernation during the winter, it is of great importance for them to absorb as much heat as possible during the short summer. He placed several species of lepidopterous larvae upon a snow surface exposed to bright sunshine. The snow melted at different rates under the various larvae, and in two hours the darkest insect had sunk by far the deepest into the snow, proving that it was the best absorber of heat. This ingenious experiment of Lord Walsingham should be made the beginning of an extensive and careful research.

Chapman ('88) has shown that it may be of advantage to moths inhabiting wet regions to display dark colors, or become melanic. His observations were made upon Diamea flagella, and he says that upon one showery afternoon he observed that one side of the tree trunks was wet and dark in color; the other side being dry was paler. "As a consequence, the dark specimens of flagella were very conspicuous upon the dry portions, hardly visible on the wet, whilst with the ordinary form the conditions were reversed, those on the wet bark were conspicuous, those on the dry much less so." Perhaps the dull coloration of Arctic moths may be partially due to the effect of the somber background of rocks in the regions which they inhabit.

(6) *Relation between Climate and Colors of Papilios.* It is well known that the Lepidoptera in the Tropics display the richest variety and greatest number of colors. I have counted the colors exhibited by the 22 species of Papilio enumerated by Edwards as inhabiting North America north of Mexico, and also those which are displayed by the 200 species of Papilio named in Schatz's list as found in South America. The "colors" were determined by comparison with the colored plates in Ridgway ('86).

In this manner it was determined that the North American Papilios exhibit 17 colors, viz., black, brown, primrose-yellow, canary-yellow, sulphur-yellow, orange, white, greenish white, apple-green, cream-color, azure-blue, sage-green, rufous, pearl-gray, indigo-blue, iridescent blue, iridescent green.

On the other hand the South American Papilios exhibited 36 colors, viz., black, translucent black, brown, white, canary-yellow, citron-yellow, olive-yellow, primrose-yellow, chrome-yellow, straw-yellow, gamboge-yellow, cream-color, greenish white, apple-green, malachite-green, emerald-green, sage-green, slaty green iridescence, pea-green, azure-blue, iridescent Berlin-blue, indigo, pearl-blue, glaucous blue, salmon-buff, écru-drab, flesh-color, coral-red, rose-red, vermilion, rufous, geranium-red, geranium-pink, olive-buff, iridescent geranium-pink (as in P. zeuxis), and transparent areas.

As 200 species in South America display but 36 colors, while 22 in North America show 17, it follows that, while the number of species in South America is 9 times as great as in North America, the number of colors displayed is only a little more than twice as great. The richer display of colors in the Tropics, therefore, *may* be due simply to the far greater number of species, which gives a better opportunity for color-sports to arise, and not to any direct influence of the climate. The number of broods, also, which occur in a year is much greater in the Tropics than in the Temperate Zones, so that the Tropical species must possess a correspondingly greater opportunity to vary.

V. THE CAUSES WHICH HAVE LED TO THE DEVELOPMENT AND PRESERVATION OF THE SCALES OF THE LEPIDOPTERA.

(1) *Experiments and Theory.* It is well known that the scales of Lepidoptera are morphologically identical with hairs. Indeed, a graded series from simple hairs, such as are found covering the body-surface of most Arthropods, up to perfectly developed flat scales bearing well differentiated striae may usually be found upon one and the same insect.

It is also remarkable that the color-bearing scales of beetles have been developed in the same manner as those of moths and butterflies, and that in this case also hairs have become differentiated into scales which are precisely similar in appearance to those of the Lepidoptera (see Dimmock, '83).

This is only another of the numerous instances met with in nature where similar conditions of selection have developed complex organs which are similar in appearance, though found in widely separated groups. A list of papers relating to the development of scales has been given by Dimmock ('83, p. 1–11).

Most of the hairs which cover the body-surface in Arthropods are true sensory structures, the axis of each of which is a protoplasmic process from a single cell of the hypodermis, which lies below the cuticula. They have probably been developed because the cuticula, being hard, chitinous, and inflexible, would serve but poorly as a tactile or sensory surface.

Of course no one would venture to ascribe any sensory function to the scales which cover the wing-membranes of the Lepidoptera. We may, however, make several more or less reasonable hypotheses concerning the probable uses of the scales, and by testing these suppositions arrive perhaps at some plausible explanation of their retention and the complex development which they have undergone.

(1) They may have caused the wings of the ancestors of the Lepidoptera to become more perfect as organs of flight, by causing the frictional resistance between the air and the wing-surface to become more nearly an optimum.

(2) The appearance and development of the scales may have served, as Kellogg ('94) has suggested, "to protect and to strengthen the wing-membranes."

(3) The present development of the scales may be due to the fact that they displayed colors which were in various ways advantageous to the insects.

Concerning the first of these three hypotheses, the wing has, broadly speaking, two chief functions to perform in flight. It must beat more or less downward against the air, and must, in addition, glide or cut through the air, supporting the insect in its flight. For the mere beating against the air a relatively *large* co-efficient of friction between the air and the wing might be advantageous; but for gliding and cutting through the air a *small* co-efficient of friction would certainly be an advantage. There must therefore be an optimum co-efficient of friction, which lies somewhere between these two.

In order to determine the co-efficient of friction between the wing and the air, use was made of a method which, in one form or another, has long been known to engineers; that is, of observing the ratio of damping of the vibrations of a pendulum.

It is well known that when a pendulum is swinging free, and uninfluenced by any frictional resistances, the law of its motion is expressed by the formula,

$$(1)\quad d = A \sin \frac{2\pi}{T} t$$

where d is the displacement of the pendulum from its middle position after the interval of time t, A is the maximum displacement and T the time of a complete vibration, back and forth. If, however, frictional resistances interfere, the formula becomes,

$$(2)\quad d = A\,e^{-Kt}\,\sin\frac{2\pi}{T_1}\,t$$

$$(3)\quad \text{Hence, } K = \frac{-\log d\,T_1}{A\log e\sin 2\pi\,t^2},\ \text{or if } t = T_1$$

$$(4)\quad K = \frac{-\log d}{AT_1\log e}$$

where K is a constant dependent upon the friction, e is the base of the Napierian system of logarithms and T_1 is the time of a complete vibration, which may be different from the T, representing the time of vibration when not under the influence of friction.

The plan was, then, to attach the wing of some large butterfly or moth to the end of a short, light pendulum in such a way that it would either fan against the air, or cut through it, and then to observe the ratio of damping of the pendulum's vibrations. A drawing of the pendulum with a wing attached is given in Plate **1**, Fig. 3. The wing is here shown in the position for "cutting or gliding" through the air. It would be in the position for fanning against the air, if it were rotated 90°. The pendulum was made of brass and steel, the ends being of brass and the slender middle portion of steel. Its vibrations were read off upon an arc graduated in millimeters. The readings were certainly accurate down to 0.5 mm. The pendulum was hung upon a steel knife edge (N, N, Fig. 3), which rested upon firm level glass bearings. The pendulum was 24.21 cm. long, and weighed 19.61 grams. Its time of vibration (T_1) was 0.877 seconds. This rate of vibration was practically unaltered when a wing was fastened to the end of the pendulum, the reason being that the wings were very light, the heaviest, that of Samia cecropia, weighing only 0.038 grams. The wing to be experimented upon was fitted into a deep, narrow slot at the free end of the pendulum, and then cemented in by means of a little melted beeswax. It thus became a perfectly rigid part of the pendulum itself.

The pendulum with wing attached was deflected through a known arc, read off upon the millimeter scale, and its reading at the end of the first swing carefully observed. Then if A be the initial deflection, which we may call unity, and if d be the reading after the first swing, the ratio of damping is given by the expression $\frac{d}{A}$. In experimenting with a fore wing of Samia cecropia "fanning the air," it was found, as the mean of many trials, that this ratio of damping was 0.919, that is to say, the amplitude of the 2d swing was 0.919 as great as the amplitude of the 1st, that of the 3d only 0.919 as great as that of the 2d, and so on. The scales were then carefully removed from the wing-membranes, by means of a camel's hair brush, and by again testing the vibrations it was found that the new ratio of damping was 0.917. This is so near the value of the ratio of damping with the scales on (0.919), that it may be considered identical, the difference being due to errors of experimentation.

Hence we must conclude that the presence of the scales upon the wing-membrane has not altered, appreciably, the co-efficient of friction which would exist between scaleless wing-membranes and the air. The results indicate rather, that when the scales appeared upon the wings of the scaleless, clear-winged ancestors of the Lepidoptera, the co-efficient of friction remained unaltered. This tempts one to the further conclusions, that the co-efficient of friction between the air and the wings was already an optimum in these clear-winged ancestors before the appearance of the scales, and therefore that Natural Selection would operate to keep it unaltered.

A wing of Samia cecropia cut so as to give it the same shape and dimensions as one of Morpho menelaus, gave an identical damping ratio. I conclude that the co-efficient of friction may be the same for both moths and butterflies, at least for those which move their wings at about the same rate in flight.

It was found in the case of the Samia cecropia wing, that when it was vibrated in the position for "cutting through" the air, the ratio of damping was 0.991. It will be remembered that, when the wing "fanned" the air, this ratio was 0.917. We may find the ratio between the resistance encountered in "fanning" and that encountered in "gliding" through the air by substituting these values in equation (*4*), $K = \frac{-\log d}{A T_1 \log e}$.

Thus for fanning, $\frac{d}{A} = 0.917$ and $T_1 = 0.877$. Making A unity,

$$K = \frac{-\log 0.917}{0.877 \log e} = 0.1.$$

In cutting through the air, $\frac{d}{A} = 0.991$ and T_1 as before $= 0.877$.

Hence in this case $K = \frac{-\log 0.991}{0.877 \log e} = 0.01.$

The wing, then, encounters at least 10 times the resistance in fanning that it does in gliding through the air. It should be said that this last experiment is somewhat crude, for the wing necessarily could not be made to cut the air with that delicate precision which is probably realized by the insect in flight. I should not be surprised, if in nature the insects encountered at least 20 times the resistance in beating the air, that they do in merely gliding through it.

Concerning Mr. Kellogg's supposition, that the scales were developed to "protect and to strengthen the wing-membranes," I will admit that they may serve in some slight degree to protect the wing-membranes from scratches, etc.; but I am unable to accept his conclusion, that they strengthen the wing-membranes, any more than that the shingles upon a roof serve to add strength to it. The wing-membranes themselves are tough, elastic, and not easily torn or scratched, and the scaleless wings of the Neuroptera and Hymenoptera are very rarely found torn or scratched in nature.

In 1858 Mr. Alexander Agassiz called attention ('59) to the fact, that "the nervures of the wings of butterflies are so arranged as to give the greatest lightness and strength; they are hollow, with their greatest diameter at the base of the wing, the point of greatest strain, their diameter gradually diminishing to the edge of the membrane. If a section be made across such a wing parallel to the axis of the body, we find very much the arrangement which has been experimentally proved by Fairbain and Stephenson as giving the greatest strength of beams, as exemplified in the tubular bridge. We find the strongest nervure placed either on or near the anterior edge of the upper wing; there is no such nervure on the lower wing, all being of nearly the same size, as such a one would have prevented the elasticity of the wing from assisting the flight to any considerable extent." Mr. Agassiz has informed me that he carried out an extensive series of experiments upon the rigidity of the wings of various species of Lepidoptera. He placed little platinum strips upon the wings and observed the extent of the bending produced. His results demonstrated that the Sphinx moths possess by far the strongest wings, and that the Danaoid and Acraeoid Heliconidae have very weak wings. The reason for this probably lies in the fact, that the Sphinx moths move their wings with great rapidity, while, according to Bates ('62) and all subsequent observers, the Heliconidae have a slow flight.

As the scales have been developed not because they aided the insects in flight or strengthened the wings, their retention must have been due to some other cause, probably to their displaying colors which were advantageous to their possessors in various ways. As Dimmock ('83) says, "it is only in insects where certain kinds of brilliant coloration have been developed that one finds scales." Indeed, I believe that the vast majority of the scales found in Lepidoptera are merely color-bearing organs. They probably first made their appearance upon small areas of the wings, perhaps adjacent to the body, and were merely colored hairs, similar to those of the surface of the body, which had grown out upon the wings. In this position they displayed some color which was of advantage to the insect; perhaps serving to render it less conspicuous than formerly. Under these circumstances they would naturally be preserved through the operation of selection until finally they became modified into true scales; just as the hairs in the Coleoptera have undergone a similar modification. If this be true, it is easy to see how they might spread out over the surfaces of the wings until the whole wing became covered with scales.

(2) *Summary of Conclusions.* The scales do not aid the insects in flight, for the wings have precisely the same efficiency as organs of flight when the scales are removed. The phylogenetic appearance and development of the scales upon the scaleless ancestors of the Lepidoptera did not in the least alter the efficiency of their wings as organs of flight. This efficiency of their wing surfaces was probably, therefore, already an optimum before the scales appeared. The scales do not appreciably strengthen the wing-membranes, that function being performed by the nervures. The majority of the scales are merely color-bearing organs, which have been developed under the influence of Natural Selection.

PART B.

COLOR-VARIATIONS IN THE HELICONIDAE.

I. General Causes which determine Coloration in the Heliconidae.

In 1861, after eleven years of study within the forests of South America, Bates read his, now classic, paper upon the life and habits of the Heliconidae of the Amazon region. In it he first brought forward his ingenious theory of Mimicry—a theory which, under the able interpretations of Wallace and Fritz Müller, and in more recent times, under the impetus of the zeal of their numerous disciples, has yielded so much that is of interest to scientific men.

The Heliconidae are, above all, creatures of the forest, and Bates found that the number of species increases as one travels inland from the Lower Amazons towards the eastern slopes of the Andes, so that the hot Andean valleys near Bogota, or in Ecuador, contain perhaps the greatest number. In their range they are restricted to the Tropics of the New World. Only two species, Dircenna klugii and Heliconius charitonius, extend so far north as the extreme Southern States of the United States, and none of them are found much further south than 30° S. Lat.

Bates and Felder first saw that the Heliconidae were naturally divided into two distinct groups. One, the Danaoid Heliconidae, consists of about twenty genera, all more or less closely related, and evidently an offshoot from the great universal family, the Danaidae, members of which are found in both Hemispheres. The other group, the true Heliconidae, is composed of two closely related genera, Heliconius and Eueides. They are allied in structure to the Acraeidae and hence their name, Acraeoid Heliconidae. Schatz and Röber ('85–'92, p. 105) say of the Acraeoid Heliconidae: — They are an offshoot of the great family Nymphalidae, which have undergone a remarkable development in the length of the fore wing, and in this respect have been developed in a direction parallel with the Danaoid Heliconidae. In their structure, however, they are quite distinct from the Danaoid group.

Schatz has proposed a new classification for the Heliconidae. He finds that the genera Lycorea and Ituna, which Bates included among the Danaoid Heliconidae, are very closely allied to the Danaidae, he therefore says that Lycorea should be placed among the Danaidae, while Ituna is clearly midway between the Danaidae and the Danaoid Heliconidae. Schatz proposes the name "Neotropidae" for the Danaoid Heliconidae. However, I think the name "Danaoid Heliconidae," being older and more descriptive of their relationship, should by all means be retained. In this paper I shall follow Bates's classification, and include among the Danaoid Heliconidae the twenty genera: Lycorea, Ituna, Athesis, Thyridia, Athyrtis, Olyras, Eutresis, Aprotopos, Dircenna, Callithomia, Epithomia, Ceratinia, Sais, Scada, Mechanitis, Napeogenes, Ithomia, Aeria, Melinaea, and Tithorea. The Acraeoid Heliconidae will then consist of the two remaining genera, Heliconius and Eueides.

Staudinger ('84–'88) records 453 species belonging to the Danaoid group, and 150 belonging to the Acraeoid group.

Nearly all that we know concerning the early stages of the Heliconidae is due to Wilhelm Müller ('86). He gives figures and more or less complete descriptions of the early stages of Dircenna xantho, Ceratinia eupompe, Ithomia neglecta, Thyridia themisto, Mechanitis lysimnia, and also of Heliconius apseudes, H. eucrate, H. doris, Eueides isabella, E. aliphera, and E. pavana. Bates ('62; p. 596) says that he raised the larvae of Heliconius erato and Eueides lybia. Schatz and Röber ('85–'92) figure the larva and pupa of Ceratinia euryanassa. Edwards has given a detailed account of the early stages of H. charitonius.

Müller found that the larvae of the Danaoid group feed on various species of Solanum, while the genera Heliconius and Eueides feed upon the Passifloreae. The larvae are conspicuously colored, and often gregarious; they seem to take but little pains to hide themselves during the chrysalis stage, for Müller says that he has seen the silver-spotted, white chrysalids of Heliconius doris hanging in great numbers in the near neighborhood of the larval food plant. The mature insects also furnish a good example of what Wallace ('67) designated as "warning coloration," for their tawny orange and black wings are very conspicuous as they sail slowly around in circles, settling at frequent intervals in their lazy irregular flight.

Bates was the first to call attention to the circumstance that they often possess a rather strong and disagreeable odor, and in 1878 Fritz Müller confirmed this observation for a number of the Heliconidae. He found, for example, that the genera Ituna and Ilione have a pair of finger-like processes near the end of the abdomen, which can be protruded and then emit a rather disagreeable odor; and he also found that the Acraeoid Heliconidae, especially the females, possess a disgusting odor. Seitz ('89), however, examined about fifty species of Heliconidae and found that many of them appear to have no odor. For example, he says that Heliconius eucrate and Eueide dianasa have no odor, but that *some specimens* of Heliconius beskei, and Eueides aliphera have a horrid odor.

Whether they are odorous or not, it would seem that the Heliconidae have but few enemies to fear, for not one of the many skilled observers who have studied them in their native haunts has ever seen a bird attack them, and the only ground for believing that they are attacked rests upon the rather dubious evidence of a few specimens found by Fritz Müller having symmetrical pieces apparently bitten out of the hind wings. Belt ('74) observed that a pair of birds which were bringing large numbers of dragon-flies and butterflies to their young never brought any of the Heliconidae, although these were abundant in the neighborhood. In fact, Belt was able to discover only one enemy of these butterflies, and that was a yellow and black wasp, which caught them and stored them up in its nest to feed its young. The Heliconidae then, in spite of their weak structure, conspicuous colors, and slow flight, enjoy a peculiar immunity.

As is well known, Bates ('62) first called attention to the fact that the Heliconidae were "mimicked" or imitated both in color-pattern and shape of wings by a number of other genera of butterflies and even moths. Bates had no difficulty in showing that this mimicry might easily be explained upon the ground that the Heliconidae, on account of their bad taste and smell, were immune from the attacks of birds and other insectivorous animals, and that therefore it gave a peculiar advantage to a butterfly belonging to any other group not thus protected, to assume the shape and coloration of the Heliconidae; for then the birds could not perceive any difference between it and the true Heliconidae. Bates found that fifteen species of Pieridae belonging to the genera Leptalis and Euterpe, four Papilios, seven Erycinidae, and among diurnal moths three Castnias and fourteen Bombycidae imitate each some distinct species of the Heliconidae occupying the same district. He also found that all of these insects were much rarer than the Heliconidae which they imitated. In some cases, indeed, he estimated the proportion to be less than one to a thousand. Wallace ('89, p. 265), who has added so much to our knowledge of this subject, aptly defines this kind of mimicry as an "exceptional form of protective resemblance."

But by far the most remarkable discovery made by Bates was the fact, that species belonging to different genera of the Heliconidae themselves mimic one another. Neither Bates nor Wallace was able to give any satisfactory explanation of the cause of this latter form of mimicry, for all of the genera of the Heliconidae are immune. They therefore supposed it to be due to "unknown local causes," or similarity of environment and conditions of life.

Thus the matter rested until 1879, when Fritz Müller brought out his well-known paper upon "Ituna and Thyridia, a remarkable example of mimicry," in which he showed that both of these genera are protected, yet they mimic each other. He also showed that this mimicry might be due to Natural Selection brought about in the following manner. It is possible that young birds, upon leaving the nest, are not furnished with an unalterable instinct which tells them exactly what they should and should not eat; so they may try experiments, and would then in all probability taste a few of the Heliconidae before finding out that they were unfit to eat. Müller then demonstrated that, if this supposition be true, it becomes a decided advantage to the various species of Heliconidae to resemble one another. His reasoning was as follows: Let it be supposed that the young and inexperienced birds of a region must destroy 1,200 specimens of any distasteful species of butterfly before it becomes recognized as such, and let us assume further that there are in existence 2,000 specimens of species A, and 10,000 of species B; then, if these species are *different* in appearance, each will lose 1,200 individuals, but if they resemble each other so closely that they cannot be distinguished apart, the loss will be divided pro rata between them, and A will lose 200, and B 1,000; therefore A saves 1,000 or 50% and B saves only 200 or 2% of the total number of individuals in the species; hence, while the relative numbers of the two species are as 1 to 5, the relative advantage derived from the resemblance is as 25 to 1.

Blackiston and Alexander ('84) have given a complete mathematical statement of Müller's law, and have come to the conclusion that, if the number of individuals destroyed is small compared with the number constituting the species, the relative advantage is inversely as the square of the original numbers; but if the number destroyed is large compared with the original number, the ratio of advantage is much greater than the inverse squares of the original numbers. Their deduction may be briefly stated as follows: —

Designation of Species	A	B
(1) Original number	$a >$	b
(2) Number lost without imitation . .	e —	$= e$
(3) Remainders without imitation . .	$(a-e)$	$(b-e)$
(4) Number lost with imitation . . .	$\frac{a}{a+b}e$	$\frac{b}{a+b}e$
(5) Remainders with imitation	$a\left(1-\frac{e}{a+b}\right)$	$b\left(1-\frac{e}{a+b}\right)$
(6) Excess of remainders due to imitation, or "absolute advantage" (3)—(5) . . .	$\frac{b\,e}{a+b}$	$\frac{a\,e}{a+b}$
(7) Ratio of excess to remainders without imitation (6): (3), =proportional advantage.	$\frac{e}{a+b}\times\frac{b}{a-e}$	$\frac{e}{a+b}\times\frac{a}{b-e}$
(8) Ratio of proportional advantage of B to proportional advantage of A.	$=\frac{a\,(a-e)}{b\,(b-e)}=\frac{a^2}{b^2}\;\frac{\left(1-\frac{e}{a}\right)}{\left(1-\frac{e}{b}\right)}$	

It is evident, then, if e be small compared with a and b, that the proportional advantage of B is to the proportional advantage of A as a^2 is to b^2. If, however, the loss (e) is great compared with a or b, the relative gain for the weaker species becomes even greater than the ratio of the squares of b and a.

If it be true, then, that young birds, when they leave the nest, do *not* possess a directing instinct telling them what they should and should not eat, but actually do experiment to some extent upon various insects which they meet with, Müller's law is amply sufficient to account for the numerous cases of mimicry and remarkably close resemblances which are found among the species of the Heliconidae themselves.

Unfortunately no *direct* experiments have ever been made upon the feeding-habits of young South American birds, nor have the contents of their stomachs been examined. There have been a few experiments, however, which seem to support the idea that some animals do learn to associate an agreeable or disagreeable taste with the coloration and appearance of their prey. It is well known that Weismann ('82, p. 336-339) found that the black and yellow larvae of Euchelia jacobaeae were refused by the green lizard of Europe. He then introduced some young caterpillars of Lasiocampa rubi, which are very similar in appearance to those of Euchelia. The lizards first cautiously examined the larvae, and finally ate them. After this Weismann reintroduced the E. jacobaeae larvae and the lizards were seen to taste them, apparently mistaking them for the edible L. rubi caterpillars.

Poulton ('87) carried out a most careful and well-conducted research upon the protective value of color and markings in insects in reference to their vertebrate enemies. He experimented upon three species of lizards and a tree-frog. Poulton combines his results with those of other observers and presents them in the form of a table, which certainly supports the suggestion of Wallace ('67), that brilliant and conspicuous larvae would be refused as food by some at least of their enemies. Poulton also shows that a limit to the success of this method of defence (conspicuous larvae having unpleasant taste or smell) would result from the hunger which the success itself tends to produce. In the Tropics, indeed, where insectivorous birds and lizards are far more numerous than with us, and where competition for food is great among them, "we may feel sure that some at least would be sufficiently enterprising to make the best of unpleasant food, which has at least the advantage of being easily seen and caught." This last suggestion of Poulton certainly seems reasonable; moreover, it has occurred to me that young birds, being but little skilled in the art of obtaining their food, might quite often be forced by hunger to try various kinds of insects, and perhaps even the Heliconidae themselves.

Beddard ('92, p. 153–167) reports the results of an extensive series of experiments carried out by Mr. Finn and himself upon marmosets, birds, lizards, and toads. He arrives at conclusions which are quite different from those of Poulton and others, but it appears to me that his experiments were by no means so critically performed as those of Poulton. He frequently threw larvae into a cage containing many birds and observed them struggle for the prey. It may well be, however, that a bird would be quite willing to swallow a very unsavory mouthful in order to prevent any of its companions from, apparently, enjoying it. However, Beddard found that toads will eat any insect without hesitation in spite of brilliant coloration, strong odors, or stings. He also found that birds and marmosets would often devour "conspicuously colored" larvae without any hesitation, and that some "protectively colored" or inconspicuous larvae were refused. There can be no doubt that many insectivorous animals pay but little attention to the colors of their prey; for example, it is well known to anglers that trout and salmon will snap at the most gaudily colored "flies," which may or may not have any counterpart in nature.

The whole question of warning coloration will have to be made the subject of an extensive research upon both old and young insectivorous animals before we can safely arrive at any certain conclusions respecting it.

II. Methods Pursued in Studying the Color-Patterns of the Heliconidae.

No comparative study of the color-patterns displayed by the Heliconidae has ever been made. In fact, very few such studies have been carried out upon any Lepidoptera. The only works I know of are those of Eimer ('89) and Haase ('92) upon the coloration of the Papilios, and of Dixey ('90) upon the wing-markings of certain genera of the Nymphalidae and Pieridae. The family of the Heliconidae with its numerous species and comparatively simple coloration affords an excellent opportunity for such a research.

In making this study of the Heliconidae I was permitted through the kindness of Mr. Samuel Henshaw to make free use of the collection in the Museum of Comparative Zoölogy at Harvard. I also found the colored figures in the works of the following authors of great service: Hewitson ('56–'76), C. und R. Felder ('64–'67), Hübner ('06–'25), Humboldt et Bonpland ('33), Cramer (1779–'82), Staudinger ('84–'88), Godman and Salvin ('79–'86), and Ménétriés ('63); likewise the following shorter papers published in various serials: Bates ('63, '65), Butler ('65, '69, '69–'74, '77), Druce ('76), Godman and Salvin ('80), Hewitson ('54), Snellenen van Leeuwen ('87), Srnka ('84, '85), Staudinger ('82), and Weymer ('75, '84). I was thus enabled to examine the color-patterns of 400 (89%) of the species of the Danaoid group, and of 129 (86%) of the Acraeoid group, either from the insects themselves or from figures given by the authors named above. The remaining species were either inaccessible to me, or were so vaguely described as to be unavailable. A list of the species known to me is given in Table 28.

(1) *The Two Types of Coloration in the Danaoid Heliconidae.* It is very remarkable that the color-patterns of all of the Heliconidae

may be grouped into two very closely related types. To the one of these I have given the name "*Melinaea type*," for it is characteristic of most of the species of the genus Melinaea. It is well represented by Figs. 46, 48, 49, 51, and 55–57 (Plate **4**). The insects which belong to this type possess wings colored with *rufous*, *black*, and *yellow*.

The other type I designate as the "*Ithomia type*," for it is very characteristic of most of the species of the genus Ithomia. Figs. 47 and 52 (Plate **4**) afford examples of it. This type differs from the Melinaea in that the rufous and yellow areas upon the wings have become *transparent*.

There are, also, many species, found in numerous genera, which fall between these two types of coloration, for the yellow and rufous spots upon their wings have become translucent, so that one may speak of them as "translucent yellow" and "translucent rufous." These spots are, so to speak, in process of becoming transparent, but a few yellow or rufous scales still remain dusted over the otherwise clear spaces. Most of the Dircennas are good examples of this type (Fig. 54, Plate **4**).

Of the 400 species of the Danaoid Heliconidae, about 125 belong to the "Melinaea type." It is well represented by most of the species of the genera Lycorea, Athyrtis, Ceratinia, Mechanitis, and Melinaea. About 30 Ithomias and half a dozen Napeogenes also belong to it. About 160 species belong to the "Ithomia type," and of this number fully 120 belong to the genus Ithomia. The others are found in the genera Ceratinia, Napeogenes, Ituna, and Thyridia, and many of them resemble the Ithomias so closely that they are said to mimic them. About 100 species, some of which are found in almost all of the genera, are intermediate in their color-patterns between the Melinaea and the Ithomia types. The 15 remaining species are represented by Melinaea gazoria (Fig. 53, Plate **4**), Ceratinia eupompe, and a few Ithomias, such as Ithomia hemixantho. In these forms almost all color has disappeared, so that the whole wing has become of a uniform dull translucent yellow, bordered on the outer edges by a grayish black.

(2) *Detailed Description of the Melinaea Type of Coloration.* Figs. 46, 48, 49, 51, and 55–57 (Plate **4**) afford examples of this type of coloration. In these insects we find the proximal half of the central cell of the fore wing occupied by a rufous-colored area, which I call the "inner rufous." It is marked I in all of the figures.

Beyond the "inner rufous" we find a black spot, marked II in the figures. It usually occupies the middle region of the cell of the fore wing, and I have designated it as the "inner black." Beyond the "inner black," and occupying most of the outer portion of the cell of the fore wing, is a light-colored area, marked III in the figures. This area is rufous in color in Fig. 49, but it is usually yellow, as in Figs. 46, 48, 51, 54–57, and I have called it the "inner yellow." Beyond the "inner yellow," and occupying the extreme outer portion of the cell, lies the "middle black" (IV). In many species it is fused, as in Figs. 46–48, 56, 57, with the large black area, the "outer black" (VII), which occupies the greater portion of the outer half of the fore wing. Just outside of the cell beyond the "middle black" one finds a well-developed yellow area (V), the "middle yellow," and there is sometimes still another yellow patch beyond this, which is marked VI and called the "outer yellow." Finally, one often finds a row of white or yellow spots, the "marginal spots" (IX), lying very near the outer margin of the fore wing (see Figs. 47–49, 51, 54, 56). These spots are very well developed in the genera Ceratinia, Napeogenes, Ithomia, and Melinaea. One more very characteristic marking of the fore wing remains to be noticed; that is the longitudinal black stripe (VIII). It is also worthy of note that the front costal edge of the fore wing is almost always tinged with black.

The pattern of the hind wing is quite simple. The ground color is usually rufous and a "middle black" band (XI) runs across the middle of the wing. The outer edge is bordered by the "outer black" (XIII). Above the "middle black" band lies the "inner rufous" (X) of the hind wing, and below the "middle black" band one finds the "outer rufous" (XII) of the hind wing. One often finds a row of white or yellow dots within the outer black border of the hind wing, and these I designate as the "marginal spots" of the hind wing.

The *Ithomia type* of coloration, it will be remembered, may be derived from the Melinaea, by simply imagining the rufous and yellow areas to have become transparent. Also the outer black usually suffers a reduction so as to become only a rather narrow border along the outer margin of the fore wing. Thyridia psidii (Fig. 47) is a good example of this type. It will be seen that the black areas remain about the same as in the Melinaea type, but that the rufous and yellow have become transparent. The middle and outer yellow areas have also fused into a large transparent patch.

Ithomia sao (Fig. 52, Plate **4**) is another good example of the Ithomia type. In this particular species the "inner black" of the fore wing is absent, and the "middle black band" of the hind wing has disappeared. When we come to consider the other Ithomias, we shall find that in this genus it has probably fused with the marginal black of the hind wing.

I have made a record of the color-variations that affect the various characteristic areas just considered, and have recorded them for every one of the species of the Danaoid and Acraeoid Heliconidae known to me. As these records are too extensive for convenient inspection, I have condensed the results, and they will be found in Tables 1–27 inclusive. Thus, Table 1 gives the variations in color of the "inner rufous" area of the fore wing for each genus of the Danaoid Heliconidae; Table 2 records the variations of the "inner black"; Table 3 the "inner yellow" area, etc. In Table 1 we find, for example, opposite the genus Ituna, the number 2 in the column labeled "transparent." This indicates that in two species of Ituna the "inner rufous" area is transparent.

In order to facilitate the study of the color-patterns Dr. Davenport suggested that I make use of the ingenious projection method invented by Keeler ('93). This method consists in "squaring the wing" in the manner shown in Figs. 4 and 5 (Plate **1**). In Fig. 4 the large rectangle (A, B, C, D) just at the right of the figure of the *hind* wing represents a kind of Mercator's projection of the wing itself. The nervures 1^a, 1^b, 2, 3, etc., are represented by the vertical lines 1^a, 1^b, 2, 3, etc., on the rectangle A, B, C, D. In cells I^a, I^b, and I^c, (bounded by nervures 1^a, 1^b, and 2,) one finds a sinuous line winding across the middle of the cell. This line appears in the same relative position upon the rectangle A, B, C, D. The same is true of the eye-spot found in the cell bounded by nervures 2 and 3, and of all the other markings of the wings. The central cell of the wing itself is shown projected in the dotted rectangle E, F, G, H.

In the case of the *fore* wing (Fig. 5), the central cell of the wing is dotted, and is shown projected upon the similarly dotted area within the rectangle I, J, K, L. In other respects the method of projection is the same as in the case of the hind wing.

In this manner the colors displayed by various species of Danaoid and Acraeoid Heliconidae have been represented in color in Plates **5–8**. Each large rectangle upon the left hand side of the Plate represents a hind wing, the small middle rectangles show the colors of the cell of the hind wing, and the right hand rectangles give the fore wings, all being projected in the manner illustrated in Figs. 4 and 5, Plate **1**. The chief advantage in Keeler's projection method lies in the fact, that similar areas in the projection of the wings lie vertically under or over one another, and thus by merely glancing up or down the plates one may observe the color-variations which occur in homologous cells of all the species represented.

III. GENERAL DISCUSSION OF THE COLOR-PATTERNS AND OF MIMICRY IN THE GENERA HELICONIUS AND EUEIDES.

Among the species of the genera Heliconius and Eueides we find remarkably little variation in venation, but great diversity in color-pattern of the wings, and in this respect they are very different from the Danaoid Heliconidae, where, it will be remembered, we find fully twenty different types of venation and only two types of color-pattern.

(1) *The Four Color Types in the Genus Heliconius.* Schatz and Röber ('85–'92) divide the species of the genus Heliconius into four groups based on color differences, as follows:—(1) the "Antiochus group" (Plate **4**, Fig. 50); (2) the "Erato group" (Fig. 60); (3) the "Melpomene group" (Fig. 59); and (4) the "Sylvanus group," a good example of which is Heliconius eucrate (Fig. 58, Plate **4**).

It will become apparent through an inspection of Figs. 50, 60, 59, and 58, which represent respectively, Heliconius antiochus, H. erato, H. melpomene, and H. eucrate, that the first three are quite closely related in color-pattern, while the fourth (H. eucrate) approaches very closely to the plan of coloration of the Melinaea type of the Danaoid Heliconidae. In fact this resemblance is so close that it may be safely said that the members of the "Sylvanus group," to which H. eucrate belongs, mimic the Danaoid Heliconidae.

The "Antiochus group" is represented by Heliconius antiochus (Plate **4**, Fig. 50, and Plate **5**, Fig 62). H. sara, H. galanthus, and H. charitonius (Plate **5**, Figs. 61, 63, 64) are also members of this group; other examples are H. apseudes, H. cydno, H. chiones, H. hahnesi, H. sappho, H. leuce, H. eleusinus, and H. clysonymus.

These species are characterized by their blue iridescence, and the narrow yellow or white bands upon the primaries; the hind wings are pointed at the outer apex, and the venation approaches the type found in Eueides aliphera. H. ricini (Plate **5**, Fig. 66) is a good example of a form intermediate in coloration between group 1 and the "Erato group" (2).

The type of group 2 is Heliconius erato (Plate **4**, Fig. 60, and Plate **5**, Figs. 67 and 68). This group is closely allied to group 1 in its characteristics. A good connecting link between groups 1 and 3, the "Melpomene group," is H. phyllis (Fig. 65).

The third, or "Melpomene group," is represented by H. melpomene, H. callicopis, H. cybele, H. thelxiope, and H. vesta (Plate **6**, Figs. 70–74, and Plate **4**, Fig. 59). H. vulcanus, H. venus, H. chestertonii, H. burneyi, and H. pachinus are also examples of this group.

(2) *Mimicry between the Genus Heliconius and the Danaoid Group.* To Schatz's group 4, the "Sylvanus group," belong all those species of Heliconius which have departed widely from the coloration pattern of the other three groups, and have come to resemble various species of the genera Melinaea, Mechanitis, and Tithorea of the Danaoid Heliconidae. H. eucoma, H. eucrate, H. dryalus, and H. sylvana (Plate **8**, Figs. 88, 89, 91, and 95) are good examples of group 4. By glancing at the diagrams on Plate **8** it will be seen that H. dryalus resembles Melinaea paraiya very closely; in fact, the likeness is so close that it is almost certain that no eye could distinguish between the two insects when they are upon the wing. Another startling resemblance is that between H. eucrate and Melinaea thera (Plate **8**, Figs. 91 and 92); moreover, there is but little difference between the color-patterns of H. eucrate, Eueides dianasa, and Mechanitis polymnia (Figs. 91, 93, and 94). H. sylvana and Melinaea egina (Figs. 95 and 96) are also said to mimic each other. The resemblance certainly appears very close at a casual glance, yet when the colors are plotted, as in Figs. 95 and 96, the differences become quite apparent. H. claudia (Plate **5**, Fig. 69) is a good connecting link between the Sylvanus group and the Melpomene group. In both the Melpomene and Sylvanus groups the venation has departed from the Eueides aliphera type, and the contour of the hind wings is much more rounded and elliptical than is the case in the Antiochus and Erato groups. (Compare Figs. 50 and 60 with Figs. 58 and 59, Plate **4**.) There are rather less than twenty species which certainly

belong to the Sylvanus group; among them may be mentioned, in addition to those already spoken of, Heliconius numata, which resembles Melinaea mneme and Tithorea harmonia; H. zuleica, which resembles a Mechanitis and is a good copy of Melinaea hezia; and H. metalilis, which is said to mimic Melinaea lilis; there are also striking resemblances between

H. aurora and Melinaea lucifer;
H. eucrate and Mechanitis lysimnia;
H. hecuba and Tithorea bonplandii;
H. formosus and Tithorea penthias;
H. telchina and Melinaea imitata;
H. messene and Melinaea mesenina;
H. hecalesia and Tithorea hecalesina;
H. ethra and Mechanitis nesaea;
H. pardalinus and Melinaea pardalis;
H. ismenius and Melinaea messatis.

Most remarkable of all perhaps is the close resemblance between Heliconius aristiona, Mechanitis methone, and Ithomia fallax of Staudinger. In fact, Staudinger states in his "Exotische Schmetterlinge" that he hesitated for some time to describe Ithomia fallax on account of its close resemblance to Hewitson's Mechanitis methone. Good lists of the Heliconidae which are said to mimic one another are given by Wallace ('89, p. 250, 251), and by Haase ('93^{a}, p. 146, 147).

(3) *The Three Color-Types in the Genus Eueides.* In the genus Eueides we meet with three color-types represented by E. aliphera, E. thales, and E. cleobaea. These insects are distinctly smaller than the species of the genus Heliconius, and the yellow spots upon their primaries are more ocherous in color than in Heliconius. E. aliphera (Plate **6**, Fig. 77) represents the most highly specialized color-type. Eueides mereaui (Fig. 76), however, is a good connecting link between the color-patterns of E. aliphera and E. thales (Fig. 75), and E. thales is almost identical in color-pattern with Heliconius vesta (Fig. 74).

The other type of Eueides is represented by E. cleobaea, E. dianasa, E. isabella, etc. (Plate **6**, Fig. 78, and Plate **8**, Fig. 93). These resemble the Sylvanus group of Heliconius or various Melinaeas and Mechanitis.

(4) *Detailed Discussion of Plates* **5–8**. Plate **5** is intended to illustrate the types of coloration found in the Antiochus and Erato groups of the genus Heliconius. In H. sara (Fig. 61) the wings are suffused with a dark blue iridescence, and some narrow yellow bands of color are found upon the primaries. In H. antiochus (Fig. 62) we find similar bands of color upon the primaries, but they are changed to white. H. antiochus may have descended

from an albinic sport of H. sara. In H. galanthus (Fig. 63) the white areas have greatly increased in size, and the iridescent blue has become much lighter. In H. charitonius (Fig. 64) we find the wings crossed by yellow spots and bands, but in some specimens this yellow color exhibits a decidedly reddish tinge. The figure of H. charitonius in Staudinger's "Exotische Schmetterlinge" illustrates this peculiarity; indeed, spots which are commonly yellow are often found red, and *vice versa*. In H. phyllis (Fig. 65) we find along the upper part of the diagram of the hind wing a yellow marking, and a similarly shaped red mark is found in its near ally, H. thelxiope (Fig. 73, Plate **6**). The same is also true of H. ricini (Fig. 66, Plate **5**).

H. erato (Figs. 67 and 68, Plate **5**, and Fig. 60, Plate **4**) is very remarkable, for there are no less than four distinct color-types exhibited by different individuals of this species; one of them (Fig. 67) shows the basal half of the hind wing marked by six red tongues of color edged with iridescent blue, and there is a dark rufous suffusion upon some parts of the fore wing. In other specimens (Fig. 68) the red tongues of color which characterized the hind wing of Fig. 67 are almost absent, and only the blue iridescence is left; also there is no rufous to be seen upon the fore wing. In another type the blue iridescence of the hind wing has become green, and in still other specimens the yellow stripes upon the fore wing have become white.

As one looks over the diagrams upon Plates **5–8**, it becomes evident that yellow frequently changes to white, for we often find one or two species of a genus which exhibit white spots identical in shape and position with spots which are yellow in most of the others. Good examples of this are H. antiochus (Plate **5**, Fig. 62), Melinaea parallelis and Ceratinia leucania (Plate **7**, Figs. 82 and 83); likewise the white spot near the outer apex of the fore wing in H. eucrate (Plate **8**, Fig. 91), which is yellow in many individuals. Yellow areas are also frequently changed to rufous or red; thus the yellow basal half of the hind wing of H. eucrate (Plate **8**, Fig. 91) is often found of a rufous tinge in individual specimens of the species, and among the specimens of this species in the Museum of Comparative Zoölogy one can trace a gradation of this area from bright yellow to rufous. H. claudia (Plate **5**, Fig. 69) is introduced in order to exhibit some of the differences between the "Sylvanus" group, to which it belongs, and the "Antiochus" and "Erato" groups.

Plate **6** is intended to exhibit the characteristic color-patterns found in the Melpomene group and in the genus Eueides. Fig. 70 represents H. melpomene, and Fig. 71 its near ally, H. callycopis, in which the red area of the fore wing has become broken up, and some red spots have made their appearance near the base of the hind wing. In the next variety of H. melpomene, H. cybele (Fig. 72), it is remarkable that the pattern of the fore wing has come to resemble the Sylvanus type, and is identical in general plan of coloration with the fore wings of the Melinaeas or Mechanitis (see Figs. 84 or 85, Plate **7**, or Figs. 92 or 94, Plate **8**). In its close ally, H. thelxiope (Fig. 73), a still nearer approach to the Melinaea type has come about by the development of a black band across the middle of the hind wing, and one has only to imagine a general fusion of the seven club-shaped red stripes of the hind wing in Fig. 73, Plate **6**, in order to produce exactly the Melinaea type as exhibited, for example, by Eueides cleobaea (Fig. 78). In this connection it is worthy of note that Bates ('62) showed that H. thelxiope was derived from H. melpomene, there being between the two many intermediate forms.

H. vesta (Fig. 74) is evidently a close relative of H. thelxiope, and what is still more worthy of note is, that it is almost identical in the general effect of its color-pattern with Eueides thales (Fig. 75)! The yellow spots upon the fore wing of E. thales are, however, duller in hue than are those of H. vesta, and the insects are somewhat different in size, H. vesta spreading 78 mm., while E. thales spreads only 66 mm. It will be noticed that the chief difference between the color-patterns of these two species lies in the fact, that, while the black stripes of the hind wings in H. vesta lie along the nervures, in Eueides thales they occupy the middle of the cells themselves. The general resemblance of the two color-patterns may of course be merely accidental. An easy explanation, however, is afforded by the theory of mimicry, for the two species look very much alike until one subjects their color-patterns to close analysis, when remarkable differences appear. E. thales (Fig. 75) may have been derived from some such form as E. mereaui (Fig. 76), for one has merely to imagine a greater development of the black and a general deepening of the rufous upon the hind wing of E. mereaui to make it resemble E. thales quite closely. Finally, in E. aliphera (Fig. 77) the black serrated border of the hind wing is still more reduced, and the black stripe which crosses the cell of the fore wing in E. mereaui is not present.

Plate **7** is intended to illustrate the peculiarities of color-pattern found among the Danaoid Heliconidae. Thyridia psidii (Fig. 79) is an example of the transparent type of color-pattern found among the Danaoid Heliconidae, and especially prevalent among the Ithomias. It will be seen by comparing Fig. 79 with the other figures upon Plates **7** and **8**, that the chief difference lies in the fact, that in this type both the rufous and yellow areas have become transparent. The black area of the fore wing has also suffered a reduction, especially along the outer margin of the wing. Incidentally it should be mentioned, that in this *particular* species the middle black band of the hind wing has become tilted up at a sharp angle, instead of crossing the wing horizontally. A life-size figure of the wings of Thyridia psidii is given on Plate **4**, Fig. 47.

In Napeogenes cyrianassa (Fig. 80) and Ceratinia vallonia (Fig. 81) portions of the usually yellow and rufous areas have become transparent.

The spots upon the fore wing of the Melinaeas are usually yellow, but in Melinaea parallelis (Fig. 82) they are white. It would seem that this form may have descended from some albinic sport. Ceratinia leucania (Fig. 83) resembles Melinaea parallelis so closely in general plan of coloration, that it is very difficult to distinguish between them, even when the two insects are seen side by side. Ceratinia leucania, however, is somewhat smaller than Melinaea parallelis. Both occupy the same region in Central America, and the specimens from which the diagrams were drawn came from Panama.

Figs. 84–87 are drawn from various specimens of Mechanitis isthmia, all from Panama. They are intended to give some idea of the range of individual variation which is met with in this extremely variable form The contraction of the middle black band of the hind wing in this form has already been noticed in the general discussion of the laws of color-pattern (see page 184). In Fig 87 it will be seen that the inner yellow stripe which usually crosses the cell of the fore wing has become very narrow and changed to a rufous color. However, upon the under surface of the wing it still remains as a yellow stripe. Indeed, in most color-changes the upper side of the wing seems to take the initiative, the under surface being more conservative. This is not true, however, in the Ithomias, where the black areas of the under side of the wings often are found to be rufous in color, while they still remain of the normal black upon the upper surface. The colors of the under surface are, however, usually identical with those of the upper, though they are always *duller in hue.* This may be due to the fact, that the colors of the upper surface are more frequently seen than those of the lower, for these insects often float lazily along with their wings horizontally extended. The operation of Natural Selection would then be more severe with the upper surfaces than with the lower.

PLATE **8** gives an analysis of the color-patterns of some of the Heliconinae and those Melinaeas, etc., which they resemble. H. eucoma (Fig. 88) is a good example of the Sylvanus type, and with its rufous, yellow, and black wings it is certainly a wonderfully close copy of the color-pattern found so commonly among the species of the genus Melinaea of the Danaoid Heliconidae.

Heliconius dryalus and Melinaea paraiya (Figs. 89, 90) resemble each other so closely in size, shape, and coloration, that it must be impossible to distinguish between them when the butterflies are in flight; yet an analysis of their color-patterns shows that there are considerable differences between them. The shape of the yellow bands upon the fore wings is quite different; the inner black spot within the cell is double in Melinaea paraiya, and there is also a row of white spots along the margin of the fore wing.

A much closer resemblance is found between H. eucrate and Melinaea thera (Figs. 91 and 92), where the Heliconius is almost a true copy of the Melinaea.

The color-patterns of Eueides dianasa (Fig. 93) and Mechanitis polymnia (Fig. 94) are also very nearly the same. Both are common species in Brazil.

Heliconius sylvana is said by Bates and by Wallace to mimic Melinaea egina. It will be seen by reference to Figs. 95 and 96 that their color-patterns are quite different in detail, yet the insects look very much alike when placed side by side, and may easily be mistaken for each other when upon the wing. Melinaea egina is much more common than Heliconius sylvana.

IV. GENERAL DISCUSSION OF THE COLOR–PATTERNS AND OF MIMICRY AMONG THE DANAOID HELICONIDAE.

(1) *The Origin of the Two Types of Coloration.* The character of the variation in the Danaoid Heliconidae is very different from that of the genera Heliconius and Eueides, for while there is great

diversity of color-pattern and very little variation in venation among the species of the Acraeoid group, exactly the opposite condition is met with in the Danaoid group, where we find at least twenty different types of venation and only two types of color-pattern. One of these types of coloration is well exemplified by most of the Melinaeas (Fig. 48, Plate **4**), and I have therefore called it the "*Melinaea*" type. The other type is exemplified by most of the Ithomias (Figs. 47 and 52) and has been designated in this paper as the "*Ithomia*" type. In the Melinaeas, it will be remembered, we find the rufous and black wings crossed by bands of yellow; while in the Ithomias, on the other hand, the rufous and yellow areas have become transparent, often leaving the wing as clear as glass, and the black, which is so characteristic of the outer half of the wing in the Melinaea type, has shrunk away until it has come to lie along the outer margin of the wing only.

By a study of all the genera of Danaoid Heliconidae we gain light upon the question of the origin of the "Melinaea" and "Ithomia" types of coloration. As we have seen (page 198), the Danaoid Heliconidae are an offshoot from the great family Danaidae. Indeed, two of the genera, Lycorea and Ituna, are so closely related to the Danaidae that Schatz and Röber ('85–'92) propose to include them within that family. There can be but little doubt that Lycorea and Ituna are remnants of the ancestral forms which long ago shot off from the Danaidae to form the Danaoid Heliconidae; and it is interesting to note, that in these two patriarchal genera we find the two distinct types of color-pattern which are exhibited by the Danaoid Heliconidae, for all of the five known species of Lycorea are good examples of the Melinaea type (see Lycorea ceres, Fig. 46, Plate **4**), while the four known species of Ituna all exhibit the transparent, or Ithomia, type of coloration. In fact, in their color-patterns the species of Ituna remind one of gigantic Ithomias. The species of Lycorea, however, are colored very much after the pattern of the Danaidae, and indeed they have departed but little from the type of the members of the great family whence they sprang. On this account I believe that the Melinaea type of coloration, which is so characteristic of the species of Lycorea, is phylogenetically older than the Ithomia type.

In order to account for the origin of the Ithomia type, we may assume that, shortly after the primeval forms of the Danaoid Heliconidae began to segregate out from the Danaidae, the species were few and probably rare. Under these circumstances any given insect would gain but little advantage by resembling merely the general type of the coloration of its fellows. For the relative advantage gained by such imitation, according to Fritz Müller's law, increases inversely as the square of the fraction whose numerator is the actual number of the imitating form and whose denominator is the actual number of the imitated. Therefore when the insects were still rare there would be few to imitate and consequently but little advantage would be gained by the imitation. Imagine, for example, that a single insect happens to imitate the color-pattern of a group of 100, and that the advantage gained thereby is represented by the number 1; it is evident from Fritz Müller's law that, if it happened to imitate the coloration of a group of 1,000, its relative advantage would be 100 instead of 1. We see, then, that mimicry within the group of the Danaoid Heliconidae became an important factor only after the group was well established and the insects became common. During the early history of the race, then, there would be but little tendency towards conservatism of color-patterns, and when the "Ithomia" and "Melinaea" types of coloration made their appearance, they both survived and now serve as the patterns for mimicry; and this accounts very well for the remarkable fact, that there are no other types of coloration than these two to be found within the whole group with its 450 species!

(2) *Mimicry among the Danaoid Heliconidae.* The genus Ithomia with its 230 species is the dominant genus of the Danaoid group, and in nearly all of the other genera individual species are found which have departed widely from their generic type of coloration and have assumed the clear wings of the Ithomias. A good idea of how far these interesting individuals may depart from the coloration of their type may be gained by comparing Fig. 53, Plate **4**, which represents Melinaea gazoria, with Fig. 48, which represents a typical Melinaea (M. paraiya). It is evident that Melinaea gazoria is startlingly like an Ithomia both in size and coloration, although it retains the venation and generic characteristics of a Melinaea.

In Mechanitis, which is the most independent genus of the Melinaea type of coloration, all of the species are fair examples of the Melinaea type, except Mechanitis ortygia Druce, from Peru. Druce ('76) in his description of this curious little species states in astonishment that it possesses the venation of a Mechanitis, but the size and coloration of an Ithomia!

It is quite remarkable that although the genera Melinaea and Mechanitis serve as models of mimicry for the Acraeoid Heliconidae, they should themselves mimic Ithomia.

The genus Ithomia is, however, the most independent of all the genera of the Danaoid group, and I know of remarkably few good instances in which an Ithomia has apparently departed from the coloration of its type to assume the guise of the Melinaeas. One good example of such a change, however, is afforded by Ithomia fallax of Southern Peru, which resembles either Mechanitis methone or Heliconius aristiona of Colombia (see page 210). There is apparently a difficulty in ascribing this resemblance to mimicry, for the imitator and imitated do not occupy the same geographical regions.

In direct contrast with the independence of the Ithomias stands the case of the genus Napeogenes; for Godman and Salvin ('79–'86) say of Napeogenes, that nearly every species mimics some Ithomia which occupies the same district; and thus almost the very existence of the genus would seem to depend upon its mimicry of Ithomia.

It is not the purpose of this paper to discuss, in detail, the numerous interesting cases of mimicry which are believed to exist between members of the Danaoid Heliconidae. An excellent discussion of such cases, and of the relationships of the various genera, has been given by Haase ('93^a, p. 116–127).

V. QUANTITATIVE DETERMINATION OF THE VARIATIONS OF THE CHARACTERISTIC WING-MARKINGS IN THE ACRAEOID AND DANAOID HELICONIDAE.

(1) *Variations of "Inner Rufous" Areas of the Fore and Hind Wings.* Table 1 gives the color-variations which are exhibited by the "inner rufous" area of the fore wings in the Danaoid Heliconidae. This area is marked I in all of the figures upon Plate **4**. We learn from an inspection of Table 1 that this area is rufous in color in 124 species of the Danaoid Heliconidae, transparent in 152, black in 24, and that in the remainder it is more or less translucent, and of either a yellowish or rufous tinge.

Table 10 shows the variations which come over the "inner rufous" area of the hind wings of the Danaoid Heliconidae. This area is marked X in the figures upon Plate **4**. It is apparent at a glance that the variations which affect the inner rufous areas of both fore and hind wings are very similar. In order to exhibit this fact graphically, the color-variations have been laid off upon the diagram, Fig. 97, Plate **9**. The base line is marked at equal intervals with the words "rufous," "translucent rufous," "translucent, slightly rufous," "transparent," etc., and the ordinates show the number of species which exhibit the various colors, rufous, translucent rufous, etc. For example, at the point "translucent rufous" we find that the ordinate is 23; this indicates that in 23 species the area is translucent rufous in color. The points thus found upon the ordinates are successively joined by straight lines forming a zig-zag figure. The full line represents the fore wing, and the dotted line the hind wing, and it becomes clearly evident from the closeness of these two zig-zag lines that the color of the inner rufous area of the fore wing (area I, Plate **4**) is almost always sure to be identical with that of the inner rufous area of the hind wing (area X, Plate **4**). We see, therefore, that whatever color-variation affects the inner rufous area of the fore wing, this area in the hind wing is almost always affected in the same manner.

Fig. 99, Plate **9**, is derived from Tables 15 and 24, which show the color-variations in the fore and hind wings of the genera Heliconius and Eueides. It is seen that here also the colors of these two areas in both the fore and hind wings are almost always identical. We here meet with one of those interesitng physiological laws which are independent of Natural Selection, and the meaning of which remains a mystery, for surely we can see no reason on the ground of adaptation why similar areas upon both fore and hind wing should bear similar colors.

(2) *The "Inner Black" Spot.* Table 2 shows the presence or absence of the "inner black" spot in the Danaoid Heliconidae. This spot is marked II in the figures upon Plate **4**. When present, it is always black in color and is usually found occupying the middle region of the cell of the fore wing. The table shows that it is about an even chance whether it be present or not, for it is absent in 210 species and present in 190. In the genus Ithomia, however, it is present in only one third of the species. What is most worthy of note concerning it, is the fact that it almost always appears, when present, as a single spot. Indeed, it appears as a double spot in only 7 species, and 5 of these belong to the genus Melinaea. A good example of its appearance as a double spot is found in Melinaea paraiya (Fig. 48, Plate **4**). It will be remem-

bered that there are 450 species in the Danaoid group; 25 of these belong to the genus Melinaea; yet among these 25 we find 5 exhibiting this marking as a double spot. Assuming that the doubling of this spot has arisen in each species as a sport, and that such a sport is as likely to appear in one species as in any other of the Danaoid group, then the chances against five such sports appearing among the 25 Melinaeas is $\frac{450\times449\times448\times447\times446}{25\times24\times23\times22\times21}$, or about 2,830,000 to 1. Indeed, it is probable that all five of the species of Melinaea which exhibit the doubling of this spot are descendants of a *single* ancestor in which it appeared for the first time double, for the mathematical chance that one such ancestor should appear among the Melinaeas, rather than in any other genus, is evidently 1 in $\frac{450}{25}$, or one chance in eighteen. The chance against two such unrelated ancestors is, however, $\frac{450\times449}{25\times24}$, or about 336 to 1, and the chance against three is $\frac{450\times449\times448}{25\times24\times23}$, or 6,560 to 1, etc.

By reference to Table 16 we find that in the genera Heliconius and Eueides the inner black area is black or iridescent blue in all of the species of Heliconius, but absent in 5 of the 18 species of Eueides known to me. These 5 include Eueides aliphera and its allies. Now there are 150 known species of the Acraeoid Heliconidae, and 24 of these belong to the genus Eueides; so it is evident that the mathematical chance against the supposition that five sports arose independently in the genus Eueides, in which the inner black was absent, is given by $\frac{150\times149\times148\times147\times146}{24\times23\times22\times21\times20}$, or 13,900 to 1. It is therefore probable that the five Eueides lacking the inner black are the descendants of a single ancestor.

(3) *Variations of the "Inner Yellow" and "Middle Yellow" Areas.* Tables 3 and 5, and diagram Fig. 98, Plate **9**, show the color-variations of the "inner yellow" and "middle yellow" areas in the fore wings of the Danaoid Heliconidae. These areas are marked III and V, respectively, in the figures upon Plate **4**. The "inner yellow" area, it will be remembered, occupies the outer portion of the cell of the fore wing; while the "middle yellow" is found in the region just beyond the outer limits of the cell. The two areas are often fused together as in Figs. 47, 48, 50, 51, and 55, Plate **4**. The inner yellow area is usually smaller than the middle yellow, and a comparison of Tables 3 and 5 will show that it is much more frequently obliterated by the encroachment of the rufous or black

areas which surround it; for example, while the middle yellow is rufous in color in only 14 species, the inner yellow is rufous in 56; also the inner yellow area, being usually smaller and less conspicuous than the middle yellow, is less important in cases of mimicry, and the diagram Fig. 98, Plate **9**, shows that it is much more variable in color than the middle yellow. The full zig-zag line in this figure represents the color-variations of the inner yellow, while the dotted zig-zag line gives the color-variations of the middle yellow. As there are nine color-variations displayed by each of these two areas, and as there are 400 species of the Danaoid Heliconidae recorded by me, it becomes evident that, if there *were* no color preferences displayed by these areas, there would probably be about $\frac{400}{9}$, or 44.4, species which would display it as rufous, 44.4 translucent, 44.4 yellow, etc. The heavy, straight, dotted line (Fig. 98, Plate **9**) represents this ideal condition, which would be approximately realized were one color as likely to occur as another in the respective areas. Now it is evident from an inspection of the figure, that the full zig-zag line, which represents the color-variations of the "inner yellow," approaches the straight line condition more nearly than does the dotted zig-zag line, which represents the middle yellow.[1] The inner yellow is therefore more liable to color-variations than the middle yellow; and this is what we should expect on account of its comparatively small size and its consequent inconspicuousness as a characteristic marking in cases of mimicry.

A comparison of Figs. 97 and 98, Plate **9**, is interesting, for it shows that the color-variations of the inner rufous are quite similar to those of the inner yellow and middle yellow. This serves to illustrate the close physiological relationship which exists between rufous and yellow. The two pigments are probably closely related chemically, for every ordinarily rufous area is sometimes found to be yellow, and *vice versa*. Yellow areas also often change to white. Rufous, yellow, and white are evidently closely related color-variations.[2]

[1] This is not true for one color, white.

[2] It may be well to mention here that the black areas upon the wings are subject to very little color-variation. In some cases, however, especially upon the under surfaces of the wings in Ithomia, the black has changed to a rufous or russet color. For example, Table 4 shows that the middle black area (IV in the figures upon Plate 4) is rufous in only 12 species out of the 400 which are recorded, and all of these 12 are Ithomias. Also Tables 7 and 13 show that the outer black of the fore wing, and the outer black of the hind wing are russet in 22 and 11 species, respectively. Evidently, black is a far more conservative color than rufous, yellow, or white. Probably black is also quite different from the other pigments chemically.

Tables 17 and 19 show the color-variations affecting the "inner yellow" and "middle yellow" areas of the fore wing in Heliconius and Eueides. There is but little difference between the two tables, except that in 15 species of Heliconius the inner yellow is suffused with black or blue, while the middle yellow is never suffused by the outer black which surrounds it. Fig. 100, Plate **10**, exhibits graphically the color-variation of these two areas. The "inner yellow" is represented as a full line, and the "middle yellow" as a dotted zig-zag. It is evident that here also the inner yellow is more variable in color than the middle yellow, for not only does the inner yellow area display two more colors, but its chart is a flatter zig-zag.

(4) *Variations of the "Middle Black" Mark of the Fore Wing.* Table 4 shows the color-variation of the middle black mark (area IV in figures upon Plate **4**). This marking lies along the extreme outer border of the central cell of the fore wing. It is small in area, but is rendered very conspicuous from the fact that it is situated between the inner yellow and middle yellow markings. In spite of its small size, however, it is a remarkably permanent marking, for Table 4 shows that it is absent in only 20 out of 400 Danaoid Heliconidae. In these 20 it has been obliterated by the fusion of the inner and middle yellow areas. It is worthy of note that in 12 Ithomias it has become rufous in color. This change to rufous is the only color-change which the black areas of the wings ever display.

Table 18 shows the variations of the middle black area for Heliconius and Eueides.

(5) *Variations of the "Outer Yellow" Area of the Fore Wing.* Table 6 shows the variations which affect the outer yellow area of the fore wings in the Danaoid Heliconidae. This area is marked VI in the figures upon Plate **4**; it lies beyond the region of the middle yellow, but is usually more or less fused with it. Table 6 is only approximately correct, owing to the difficulty in many cases of deciding whether the middle and outer yellow be really fused or not. It will be seen that in the genus Ithomia the middle and outer yellows are wholly fused in about 200 species. This is one of the marked characteristics of this very independent genus.

Table 20 shows the color-variations of the outer yellow area in Heliconius and Eueides. This marking is present in 81 and absent in 48 of the Acraeoid group. It is much more widely separated from the middle yellow than is the case in the Danaoid group.

(6) *The relative Permanency of the Black Areas upon the Fore and Hind Wings.* A study of the relative permanency of the various characteristic black markings upon the wings is of interest, for, if the generally accepted idea concerning the prevalence of mimicry within the group of the Danaoid Heliconidae be true, we should expect the most conspicuous markings to be the most permanent, for they are evidently of the most importance for mimicry. This is, however, not the case for the black markings. A good example of this fact is afforded by a comparison of the relative permanency of the black streak which extends along the extreme costal edge of the fore wing with the inner black spot (II in figures on Plate **4**). The inner black spot is certainly a more conspicuous marking than this narrow black streak along the costal edge; yet it is much more variable, for Table 2 shows that it is present in 210 and absent in 190 of the 400 Danaoid Heliconidae. In other words, it is about as likely to be present as absent. The black streak upon the costal edge, on the other hand, is much more permanent, for it is absent in only 52 species out of the 400.

Another good example of the inaccurracy of the supposition that large and conspicuously colored areas are always less variable than small ones, is derived from a comparison of the relative variability of the large outer black of the fore wing with the small outer black of the hind wing. Although the outer black area of the fore wing is usually much larger and more conspicuous than the outer black margin of the hind wing, it is more variable in color, for it is rufous in 22 species, while the outer black of the hind wing is rufous in only 11, out of the 400.

In general, however, large colored areas are more permanent than small ones, as was found in the case of the inner and middle yellow areas (see page 220). Indeed, a good instance of this greater variability of small color areas is afforded by the longitudinal black stripe marked VIII in the figures of Plate **4**, for this is more variable than the larger outer black area of the fore wing.

(7) *The "Middle Black Stripe" of the Hind Wing.* In the genus Ithomia the middle black stripe (XI, Plate **4**) has migrated downward, so that in many species it has become fused with the outer black margin, as in Ithomia sao (Fig. 52, Plate **4**). In other cases there is still to be seen a narrow line of rufous color between the middle black band and the outer black margin of the hind wing. Such is the case in Ithomia nise (Fig. 54, Plate **4**). In

many other cases the outer black and middle black are completely fused, so far as the upper surface of the wings is concerned; but, if one examines the under surface of the hind wings, it will be found that a narrow rufous streak still persists between the middle black band and the outer black margin of the hind wing.

(8) *Variations of the Marginal Spots of the Fore Wing.* The marginal spots are found very near the outer margin of the fore wing; they are usually either yellow or white, but in some few cases they are rufous. It appears from Table 9 that they are present in 146 and absent in 254 species of the 400 Danaoid Heliconidae known to me. Fig. 101, Plate **10**, shows graphically the manner in which these spots occur in those species which possess them. It is evident from this curve that the number of these spots is not determined merely by chance, for they show a marked tendency to appear either as 2 or 3, or as 6 or 7 spots. It is due to this fact, that there are two maximum points upon the diagram Fig. 101, Plate **10**. In those species which exhibit the "2- or 3-spot" condition, the spots are found near the front apex of the fore wing. In the "6- or 7-spot" condition they lie all along the outer margin of the fore wing, one spot in each cell. In the genera Ithomia, Napeogenes, and especially in Ceratinia these marginal spots have become large and conspicuous ornaments. (See Fig. 49, Plate **4**.)

Table 22 shows the manner of appearance of these spots in the genera Heliconius and Eueides. They are found in only 26 species of the 129 known to me; and this number is far too small to warrant general conclusions concerning the order of their appearance.

(9) *The Marginal Spots of the Hind Wing.* Table 14 illustrates the manner in which the marginal spots of the hind wings make their appearance. They are absent in 279 and present in 121 of the 400 species of the Danaoid group. Thus they occur rather less frequently than the marginal spots of the fore wing. In the 121 species in which these spots are found they show a decided tendency to appear either as 4 or as 5 spots. Fig. 102, Plate **10**, is a graphic representation of the distribution of these spots, derived from Table 14. It appears that the outline of the figure approaches a probability curve, and is approximately symmetrical about the mean ordinate (A, B), situated at 4.54.

VI. Comparison of the Color-Variations of the Papilios of South America with those of the Heliconidae.

In order to emphasize the peculiarities of the coloration of the Heliconidae, I will conclude by instituting a comparison between their variations and those of the South American Papilios. There are about 200 species of Papilio in South America, and these display in all 36 distinct colors. The colors have been determined by reference to the plates in Ridgway's "Nomenclature of color for naturalists." A list of the colors which are displayed by these Papilios has already been given upon page 191.

By exercising a very fine discrimination in distinguishing color we may count 15 distinct colors which are displayed by the 450 members of the Danaoid Heliconidae, as follows: black, brown, translucent black, sulphur-yellow, canary-yellow, citron-yellow, primrose-yellow, yellow-rufous, reddish rufous, rufous, white, translucent yellow, translucent rufous, transparent areas upon the wings, transparent areas which display iridescence. We see, then, that while the 200 species of Papilio display 36 different colors, the 450 Danaoid Heliconidae exhibit only 15. In other words, the *numbers* of the *species* and of the *colors* are almost in inverse ratio in the two groups; for while the Papilios are only $\frac{4}{9}$ as numerous as the Danaoid Heliconidae, they display almost $\frac{10}{4}$ times as many colors; and this is all the more remarkable when we remember that the general class of coloration in the Papilios and Danaoid Heliconidae is apparently the same. That is to say, in both groups we find all of the species displaying decidedly conspicuous colors, the coloration of the upper surfaces of the wings being in both rather more brilliant than that of the lower surfaces, but without essential differences in color-pattern. Nor is there an attempt in either case at protective resemblances, such as the imitation of the coloration of bark, leaves, etc. The color-patterns of the Papilios are, moreover, extremely complex, and upon comparing the different species, there are seen to be frequent fusions and obliterations of the characteristic markings, so that Haase ('93), who has made an extensive study of their color-patterns, is forced to divide them into many small groups of a few species each. The variation in the form of the wings is also very great among the Papilios, for while P. protesilaus possesses upon its hind wings, long tail-like appendages, the hind wings of P. hahneli are rounded off and without marked appendages.

There is, apparently, but one important respect in which the Danaoid Heliconidae are more variable than the Papilios, and that is size. For example, Lycorea ceres, which is probably the largest of the Danaoid group, has 2.2 times the spread of wing of Ithomia nise, which is one of the smallest (see Plate **4**, Figs. 46 and 54). The largest Papilio, P. androgea, on the other hand, spreads only 2.16 times as much as the smallest, P. triopas.

There is another minor respect in which the color-patterns of the Papilios are different from those of the Heliconidae. In the Heliconidae the fore wing slightly overlaps the hind wing, and that portion of the hind wing which is hidden from view is always dull in color (see Plates **5**–**8**). In the Papilios, however, the fore wing does not overlap the hind wing to such an extent as in the Heliconidae, and it is worthy of note that the costal edges of the hind wings in the Papilios are as brilliantly colored as are any other portions of the wings.

It is difficult to account for the remarkable conservatism in respect to color-variations among the Heliconidae, unless we resort to the explanation afforded by the theory of mimicry; for, while there is such remarkable simplicity and uniformity of color-pattern throughout the whole group of the Heliconidae, *individual variations* are very common. In the collection at the Museum of Comparative Zoölogy, for example, one finds a regularly graded series of specimens of Heliconius eucrate; at one end of this series the "inner rufous" area of the hind wing is bright yellow, and at the other end it is rufous; intermediate specimens are found in which this area is yellow, but dusted over with rufous scales. Also the "middle black band" of the hind wings in Melinaea parallelis is very variable, some specimens showing it broken in the middle (Plate **7**, Fig. 82), and others having it as an entire band. I have also seen one specimen of H. burneyi in which the commonly yellow spots upon the under surface of the wings were changed to white. Another good instance of individual variabilty is afforded by H. phyllis (Plate **5**, Fig. 65), for in this species the series of small red spots sometimes found just below the yellow band upon the hind wing is very variable, and more often absent than present. Still other instances of individual variability are seen in the yellow stripes upon the wings of H. charitonius (Plate **5**, Fig. 64), which are often found tinged with rufous. Also the remarkable diversity in Mechanitis polymnia, and M. isthmia (Plate **7**, Figs. 84–87) are other examples which show that there is no lack of individual variability among the Heliconidae. Yet the Danaoid species as a whole vary but little from the two great types of coloration represented by Ithomia and Melinaea, and in this respect they are very different from the Papilios, where we find a great many color-types and great diversity in shape of wings. Surely there must be some cause for the remarkable fact that the Danaoid Heliconidae with their 453 species should display but two types of color-pattern. I can think of but one explanation, which is afforded by Fritz Müller's theory of mimicry.

In conclusion it gives me pleasure to thank those friends whose generous aid and kindness have done so much to render the prosecution of this research a pleasure to me. I wish to express my gratitude to Dr. Charles B. Davenport, who is the real instigator and promoter of this research; to Mr. Samuel Henshaw, to whom I am indebted for numerous kindnesses, and who placed at my disposal the extensive entomological collections and library of the Museum of Comparative Zoölogy at Harvard; to Prof. Edward L. Mark for his kindness in revising the manuscript of this paper, and for the numerous valuable suggestions which he has made; to Dr. Samuel H. Scudder, to whom I am grateful for much kind advice and for the use of rare works in his library; to Prof. Ogden N. Rood for his valuable suggestions in regard to the spectroscopic apparatus; to Dr. Alpheus Hyatt for his valued and kind advice, and to my father, Prof. Alfred M. Mayer, for the use of Maxwell's discs and the direct-vision spectroscope.

PART C.

GENERAL SUMMARY OF RESULTS BELIEVED TO BE NEW TO SCIENCE.

(1) The great majority of the colors of Lepidoptera contain a surprisingly large percentage of black (p. 172).

(2) The colors displayed by the scales are not simple, but compounded of several different colors (p. 173).

(3) The pigments of the scales of Lepidoptera are derived by various chemical processes from the blood, or haemolymph, of the

pupa. The pupal blood of the Saturnidae is a proteid substance containing egg albumen, globulin, fibrin, xanthophyll, orthophosphoric acid, iron, potassium, and sodium (p. 176).

(4) In Callosamia promethea and Danais plexippus the pupal wings are at first perfectly transparent, then white, then impure yellow, excepting upon those portions which are destined to remain white in the mature wing. The mature colors then begin to appear near the central areas of the wings and *between* the nervures. Last of all, the nervures themselves become tinged with the mature colors. The central portions of the wings acquire their mature colors before the outer and costal edges, or the root of the wing adjacent to the body (p. 178, Plate **3**).

(5) The white stage in the development of color in the pupal wings represents the condition in which the scales are perfectly formed but lack the pigment which is destined to be introduced later (p. 178). (See, also, Mayer, '96, p. 230.)

(6) Dull ocher-yellows and drabs are, phylogenetically speaking, the oldest pigmental colors in the Lepidoptera. The more brilliant colors, such as bright yellows, reds, and pigmental greens, are derived by complex chemical processes and are, phylogenetically speaking, of recent appearance (p. 178). (See, also, Mayer, '96, p. 232.)

(7) While the number of species of Papilio in South America is 9 times as great as in North America, the number of colors which they display is only twice as great. Hence the greater number of colors displayed by the tropical forms may be due simply to the far greater number of the species, and not to any direct influence of the climate (p. 191).

(8) The following laws control the color-patterns of butterflies and moths: (a) Any spot found upon the wing of a butterfly or moth tends to be bilaterally symmetrical, both as regards form and color; and the axis of symmetry is a line passing through the center of the interspace in which the spot is found, parallel to the longitudinal nervures (p. 183). (b) Spots tend to appear not in one interspace only, but in homologous places in a row of adjacent interspaces (p. 183). (c) Bands of color are often made by the fusion of a row of adjacent spots, and, conversely, chains of spots are often formed by the breaking up of bands (p. 183). (d) When in process of disappearance, bands of color usually shrink away at one end (p. 184). (e) The ends of a series of spots are more

variable than the middle. This is only a special case of Bateson's ('94) law (p. 185). (f) The position of spots situated near the outer edges of the wing is largely controlled by the wing-folds or creases (p. 185).

(9) The scales in Lepidoptera do not strengthen the wings or aid the insects in flight. The vast majority of the scales are merely color-bearing organs, which have been developed under the influence of Natural Selection. The phylogenetic appearance and development of scales upon the originally scaleless ancestors of the Lepidoptera did not alter the *efficiency* of their wings as *organs of flight.* It is probable, therefore, that this efficiency was an optimum before the scales appeared (p. 197).

(10) A systematic study of the Danaoid Heliconidae demonstrates that their color-patterns can be placed in two types. Type 1, the more complex, is closely related to the coloration of the Danaidae from which the Danaoid Heliconidae sprang, and is therefore, phylogenetically speaking, the older type of coloration. This type is characteristic of the genera Lycorea, Melinaea, and Mechanitis, and I have called it the "Melinaea" type. It is characterized by the fact that the wings are rufous and black in color, and crossed by a definite system of yellow bands. Type 2, the "Ithomia" type, is characteristic of the genera Ithomia, Ituna, and Thyridia. The "Ithomia" type has been derived from the "Melinaea" by the originally rufous and yellow areas upon the wings having become transparent (p. 204).

(11) The phylogenetic origin of the "Melinaea" and "Ithomia" types of coloration can be accounted for upon the supposition, that when the species of the Danaoid Heliconidae began to segregate out from the Danaidae they were for a time rare (p. 215).

(12) A record of the characteristic markings upon the wings of the Danaoid and Acraeoid Heliconidae shows that, physiologically speaking, the colors red, rufous, yellow, and white are closely related, and that black is quite distinct from these, being the least variable color of all (p. 220).

(13) In both the Danaoid and Acraeoid Heliconidae, whatever color-variation affects that part of the *fore* wing which is adjacent to the body of the insect, almost always the same color-variation affects the homologous area of the *hind* wing in a similar manner (p. 218, and Fig. 99).

(14) The smaller yellow spots upon the wings of the Heliconi-

dae are more liable to color-variations than are the larger ones. This is what we should expect, if the theory of mimicry be true; for large spots are more conspicuous, and therefore their preservation is more important (p. 220). This rule, however, does not hold for the black markings of the wing (p. 222).

(15) The mathematical chance against five similar and independent color-sports arising in the genus Melinaea is as 2,830,000 to 1. Hence, the five Melinaeas which display the "inner black" as a double spot are probably descended from a single ancestor (p. 219).

(16) The marginal spots of the fore wing in the Danaoid Heliconidae show a marked tendency to appear either as 2 or 3, or else as 6 or 7 spots (p. 223, Fig. 101). The marginal spots of the *hind* wing show a marked tendency to appear either as 4 or 5 spots (p. 223, and Fig. 102).

(17) The 200 species of Papilio in South America display 36 distinct colors, while the 450 species of Danaoid Heliconidae exhibit only 15. Hence the numbers of the *species* and of the *colors* are almost in inverse ratio in the two groups. This may be explained by the fact, that the Danaoid Heliconidae mimic one another, while the Papilios do not (p. 224).

(18) The colors are dull upon those portions of the hind wing which are hidden from view by the overlapping fore wing (p. 225).

(19) There is no lack of individual variability among the species of the Danaoid Heliconidae; yet the species as a whole vary but little from the two great types of color-pattern represented by Melinaea and Ithomia. In order to account for this remarkable fact I am forced to resort to Fritz Müller's theory of mimicry (p. 225).

TABLE 1.

Showing the Variations in Color of the "Inner Rufous" (Area I in Figures on Plate **4**) of the *fore wing* in the Danaoid Heliconidae.

GENERA.	Rufous.	Translucent rufous.	Translucent, slightly rufous.	Transparent.	Transparent, slightly yellow.	Translucent yellow.	Yellow.	Black.	White.
Lycorea	5								
Ituna				2				1	
Athesis			2	1					
Thyridia				5					
Athyrtis	2								
Olyras	1		1	1				1	
Eutresis		2							
Aprotopos			1					1	
Dircenna	1	3		4	2	1		1	
Callithomia	1							2	
Epithomia	1	1							
Ceratinia	28	1	2	7	1			2	
Sais	5								
Scada						7			
Mechanitis	18				1		1	4	
Napeogenes	7	3	2	12		3		3	
Ithomia	29	13	15	120	26	5		3	1
Aeria						4			
Melinaea	22					2			
Tithorea	4							6	
Total	124	23	23	152	30	22	1	24	1
Excluding Ithomia	95	10	8	32	4	17	1	21	0

Note: The costal edge of the fore wing is usually black; it is rufous or brown, however, in 47 Ithomias and dull yellow in one; it is rufous in two species of Sais, in one species of Ceratinia, and in one species of Athesis. Hence it is black in 348 species and light colored in 52.

TABLE 2.

Showing the Variation (presence or absence) of the "Inner Black" (Area II) of the *fore wing* in the Danaoid Heliconidae.

GENERA.	Present.	Absent.	Remarks.
Lycorea	5		
Ituna	3		
Athesis	2	1	
Thyridia	5		
Athyrtis	2		
Olyras	4		
Eutresis	2		
Aprotopos	2		
Dircenna	8	4	
Callithomia	3		
Epithomia	2		
Ceratinia	29	12	
Sais	4	1	
Scada		7	
Mechanitis	23	1	
Napeogenes	13	17	Appears as 2 spots in 1 species.
Ithomia	72	140	Appears as 2 spots in 1 species.
Aeria		4	
Melinaea	21	3	Appears as 2 spots in 5 species.
Tithorea	10		
Total	210	190	
Excluding Ithomia	138	50	

TABLE 3.

Showing the Variations in Color of the "Inner Yellow" (Area III) of the *fore wing* in the Danaoid Heliconidae.

GENERA.	Rufous.	Translucent rufous.	Translucent, slightly rufous.	Transparent.	Translucent, slightly yellow.	Translucent yellow.	Yellow.	Black.	White, generally translucent.
Lycorea							5		
Ituna				3					
Athesis			2	1					
Thyridia				5					
Athyrtis	1						1		
Olyras						4			
Eutresis			1	1					
Aprotopos					1		1		
Dircenna		1	2	3	6				
Callithomia							3		
Epithomia						1	1		
Ceratinia	16	1	1	7	7	2	6	1	
Sais	2						2	1	
Scada						7			
Mechanitis	11						13		
Napeogenes	2		4	14	1	4	5		
Ithomia	10	11	14	124	23	9	13	1	7
Aeria							4		
Melinaea	14					4	4		2
Tithorea							6	3	1
Total	56	13	24	158	38	31	64	6	10
Excluding Ithomia	46	2	10	34	15	22	51	5	3

TABLE 4.

Showing the presence or absence of the "Middle Black" Mark (Area IV) of the *fore wing* in the Danaoid Heliconidae.

GENERA.	Present.	Absent.	Present, but changed to some color other than black.
Lycorea	5		
Ituna	3		
Athesis	3		
Thyridia	5		
Athyrtis	2		
Olyras	2		
Eutresis	2		
Aprotopos	2		
Dircenna	11	1	
Callithomia	3		
Epithomia	2		
Ceratinia	34	7	
Sais	5		
Scada	7		
Mechanitis	24		
Napeogenes	25	5	
Ithomia	194	6	12 rufous or brown.
Aeria	4		
Melinaea	23	1	
Tithorea	10		
Total	366	20	12
Excluding Ithomia	172	14	

TABLE 5.

Showing the Variation in Color of the "Middle Yellow" Band (Area V) of the *fore wing* in the Danaoid Heliconidae.

GENERA.	Rufous.	Translucent rufous.	Translucent, slightly rufous.	Transparent.	Translucent, slightly yellow.	Translucent yellow.	Yellow.	Black.	White, more or less translucent.
Lycorea							5		
Ituna				3					
Athesis			2	1					
Thyridia				5					
Athyrtis	1						1		
Olyras				1		3			
Eutresis				2					
Aprotopos				1			1		
Dircenna			2	3	2	2	3		
Callithomia							3		
Epithomia						1	1		
Ceratinia			2	6	4	5	24		
Sais							5		
Scada						6		1	
Mechanitis	4				1		19		
Napeogenes	2		3	11	4	4	6		
Ithomia	5	6	12	123	24	8	14		20
Aeria							4		
Melinaea	2			3		2	15		2
Tithorea							7	1	2
Total	14	6	21	159	35	31	108	2	24
Excluding Ithomia	9	0	9	36	11	23	94	2	4

TABLE 6.

Showing approximately the Number of Species in which the "Outer Yellow" (Area VI) of the *fore wing* in the Danaoid Heliconidae appears as a separated Marking. It is usually fused with the "Middle Yellow" Area.

GENERA.	Wholly fused with middle yellow.	Partially fused with middle yellow.	Separate.	Absent.
Lycorea		1	4	
Ituna	2	1		
Athesis	3			
Thyridia	5			
Athyrtis			2	
Olyras	1	3		
Eutresis		2		
Aprotopos		2		
Dircenna	7 ?	5 ?		
Callithomia		1	2	
Epithomia		1	1	
Ceratinia	22	16 about	3 about	
Sais			2	3
Scada			4	3
Mechanitis		1	20	3
Napeogenes	6		24 about	
Ithomia	200 about			
Aeria	4			
Melinaea	1		17	6
Tithorea			10	
Total	about 250	about 30	about 90	perhaps 20

TABLE 7.

Showing the Degree of Development of the "Outer Black" (Area VII) of the *fore wing* in the Danaoid Heliconidae.

GENERA.	Well developed over a large area of the fore wing.	Reduced to the outer margin of the fore wing.	Present, but changed to another color.
Lycorea	5		
Ituna		3	
Athesis	2	1	
Thyridia		5	
Athyrtis	2		
Olyras	3	1	
Eutresis	2		
Aprotopos	1	1	
Dircenna	5	7	
Callithomia	3		
Epithomia	2		
Ceratinia	28	13	2 partly rufous.
Sais	2		3 partly rufous.
Scada	3	4	
Mechanitis	22	2	
Napeogenes	26	4	
Ithomia	161	54	16 rufous or brown.
Aeria	4		
Melinaea	24		1 partly rufous.
Tithorea	10		
Total	305	95	22 partly rufous.

TABLE 8.

Showing the presence or absence of the "Longitudinal Black Stripe" (Area VIII) which runs parallel with the lower Edge of the *fore wing* in the Danaoid Heliconidae.

GENERA.	Present and well developed as a stripe.	Much reduced.	Absent.	Whole area suffused with black.
Lycorea	5			
Ituna	3			
Athesis	3			
Thyridia	5			
Athyrtis	2			
Olyras	3			1
Eutresis	2			
Aprotopos	1			1
Dircenna	7	3		2
Callithomia			1	2
Epithomia	1	1		
Ceratinia	37	2		2
Sais	3	1	1	
Scada	6		1	
Mechanitis	17		2	5
Napeogenes	20	6		4
Ithomia	200	6	4	2
Aeria	4			
Melinaea	14	5	5	
Tithorea	5			5
Total	338	24	14	24

TABLE 9.

Showing the Manner of Occurrence of the Marginal Spots (Area IX) of the *fore wing* in the Danaoid Heliconidae.

GENERA.	Without spots	1 spot.	2 spots.	3 spots.	4 spots.	5 spots.	6 spots.	7 spots.	8 spots.	9 spots.
Lycorea . . .	5									
Ituna	3									
Athesis . . .	3									
Thyridia . . .	4			1						
Athyrtis . . .	1							1		
Olyras . . .	3						1			
Eutresis . . .	2									
Aprotopos . .	1	1								
Dircenna . . .	11		1							
Callithomia . .	1				2					
Epithomia . .	2									
Ceratinia . .	19	1	3	1			4	12	1	
Sais	5									
Scada	4			1		1		1		
Mechanitis . .	17		1		2		1	3		
Napeogenes . .	14		1	3	1		5	3	3	
Ithomia . . .	137	2	14	14	7	16	14	8		
Aeria	4									
Melinaea . . .	13		1			2	4	3	1	
Tithorea . . .	5		1		1			2		1
Total	254	4	22	20	13	19	29	33	5	1

TABLE 10.

Showing the Color-Variations affecting the "Inner Rufous" (Area X) of the *hind wing* in the Danaoid Heliconidae.

GENERA	Rufous.	Translucent rufous.	Translucent, slightly rufous.	Transparent.	Translucent, slightly yellow.	Translucent yellow.	Yellow.	Black.
Lycorea	5							
Ituna		1		2				
Athesis			2	1				
Thyridia				4	1			
Athyrtis	2							
Olyras	3					1		
Eutresis		2						
Aprotopos	1			1				
Dircenna			8	2	2			
Callithomia	3							
Epithomia	2							
Ceratinia	23		3	6	6	1	2	
Sais	5							
Scada						7		
Mechanitis	16						4	4
Napeogenes	7	6		6	6	5		
Ithomia	31	14	12	133	14	5	1	2
Aeria							4	
Melinaea	19					4	1	
Tithorea	6							4
Total	123	23	25	155	29	23	12	10

TABLE 11.

Showing the Variations of the "Middle Black Stripe" (Area XI) of the *hind wing* in the Danaoid Heliconidae.

GENERA.	Present.	Absent.	Fused with the marginal black.	Changed color.
Lycorea	5			
Ituna	2	1		
Athesis	2	1		
Thyridia	2	2	1 partially	
Athyrtis	2			
Olyras	1	2		1 changed to translucent yellow ?
Eutresis		2		
Aprotopos	2			
Dircenna	4	7	1	
Callithomia			3	
Epithomia		2		
Ceratinia	21		20	
Sais		1	4	
Scada		7		
Mechanitis	22	2		
Napeogenes	15		15	
Ithomia	45	1	168	
Aeria		4		
Melinaea	15	9		
Tithorea	8	2		
Total	146	43	212	1 ?

TABLE 12.

Showing the Color-Variations of the "Outer Rufous" (Area XII) of the *hind wing* in the Danaoid Heliconidae.

GENERA.	Rufous.	Translucent rufous.	Translucent, but slightly rufous.	Transparent.	Translucent, but slightly yellow.	Translucent yellow.	Yellow.	Black.	White, somewhat translucent
Lycorea	5								
Ituna		1		2					
Athesis			2	1					
Thyridia				4	1				
Athyrtis	2								
Olyras	3					1			
Eutresis	1	1							
Aprotopos	1			1					
Dircenna	2	4	3	3					
Callithomia	1							2	
Epithomia	2								
Ceratinia	29	1	1	7	1			2	
Sais	5								
Scada						7			
Mechanitis	21							3	
Napeogenes	15							15	
Ithomia	44			2	1			165	
Aeria								4	
Melinaea	17						1	6	
Tithorea	5						2	2	1
Total	153	7	6	20	3	8	3	199	1
Excluding Ithomia	109	7	6	18	2	8	3	34	1

TABLE 13.

Showing the presence or absence, and Color-Changes of the "Outer Black" (Area XIII) of the *hind wing* in the Danaoid Heliconidae.

Genera.	Present.	Absent.	Changed color.
Lycorea	5		
Ituna	3		
Athesis	3		
Thyridia	5		
Athyrtis	2		
Olyras	4		
Eutresis	2		
Aprotopos	2		
Dircenna	12		
Callithomia	3		
Epithomia	2		
Ceratinia	41		
Sais	5		
Scada	7		
Mechanitis	24		
Napeogenes	30		
Ithomia	210	1	11 changed to rufous or brown.
Aeria	4		
Melinaea	24		
Tithorea	10		
Total	398	1	11

TABLE 14.

Showing the Number of the Marginal Spots of the *hind wing* in the Danaoid Heliconidae.

GENERA.	Without spots.	1 spot.	2 spots.	3 spots.	4 spots.	5 spots.	6 spots.	7 spots.	8 spots.	9 spots.
Lycorea		1					3	1		
Ituna	2	1								
Athesis				1	1		1			
Thyridia	4	1								
Athyrtis	1									1
Olyras	3		1							
Eutresis	1						1			
Aprotopos				2						
Dircenna	10		1						1	
Callithomia	2		1							
Epithomia	1					1				
Ceratinia	18	3	1	2	5	5	2	4	1	
Sais	4				1					
Scada	3				1	1	2			
Mechanitis	18		1	1	2	2				
Napeogenes	21		1	1	1	5		1		
Ithomia	164		8	6	12	9	6	6	1	
Aeria	4									
Melinaea	19				1			3	1	
Tithorea	4				2	2	2			
Total	279	6	14	13	26	25	17	15	4	1

TABLE 15.

Showing the Color-Variations of the "Inner Rufous" Area of the *fore wing* in Heliconius and Eueides.

	Rufous.	Reddish rufous.	Yellow.	Ocher.	White.	Black.	Iridescent blue.
Heliconius . .	35	8	11		2	29	26
Eueides . . .	14			1		3	
Total . . .	49	8	11	1	2	32	26

Note: The costal edge of the fore wing is always black.

TABLE 16.

Showing the Variations affecting the "Inner Black" Area of the *fore wing* in Heliconius and Eueides.

	Black.	Iridescent blue.	Rufous.
Heliconius	85	26	
Eueides	13		5
Total	98	26	5

Note: In 54 species of Heliconius the inner rufous is entirely suffused with black.

TABLE 17.

Showing the Color-Variations of the "Inner Yellow" Area of the *fore wing* in Heliconius and Eueides.

	Rufous.	Red.	Yellow.	Ocher.	White.	Black.	Iridescent blue.
Heliconius . .	11	12	54		20	12	3
Eueides . . .	6			12			
Total . . .	17	12	54	12	20	12	3

TABLE 18.

The "Middle Black" Area in the *fore wing* in Heliconius is present as a Black or Blue Marking in 99 Species and absent in 12.

It is present as a Black Mark in all 18 Species of Eueides.

TABLE 19.

Showing the Color-Variation of the "Middle Yellow" Area of the *fore wing* in Heliconius and Eueides.

	Rufous.	Reddish rufous.	Yellow.	Ocher.	White.	Black.
Heliconius .	11	12	65		23	
Eueides . .	5			12	1	
Total . . .	16	12	65	12	24	

TABLE 20.

Showing the Color-Variations of the "Outer Yellow" Area of the *fore wing* in Heliconius and Eueides.

	Rufous.	Reddish rufous.	Yellow.	Ocher.	White.	Black.	Iridescent blue.
Heliconius . .	2	1	47		24	33	4
Eueides . . .				6	1	11	
Total	2	1	47	6	25	44	4

TABLE 21.

The "Outer Black" Area of the *fore wing* in all the 111 species of Heliconius known to me is Black or Iridescent Blue.

It is Black in all 18 Eueides.

TABLE 22.

Showing the Manner of Occurrence of the Marginal Spots of the *fore wing* in Heliconius and Eueides.

	Without spots.	1 spot.	2 spots.	3 spots.	4 spots.	5 spots.	6 spots.	7 spots.	8 spots.	9 spots.
Heliconius . .	89		5	2	5	3	4	2		1
Eueides . . .	14		3				1			
Total	103		8	2	5	3	5	2		1

TABLE 23.

Showing the Variations affecting the "Longitudinal Black Stripe" of the *fore wing* in Heliconius and Eueides.

	Whole area suffused with black.	Well developed as a black stripe.	Absent (area suffused with rufous).
Heliconius	75	23	13
Eueides	2	16	
Total	77	39	13

TABLE 24.

Showing the Color-Variations of the "Inner Rufous" Area of the *hind wing* in Heliconius and Eueides.

	Rufous.	Reddish rufous.	Yellow.	Ocher.	Iridescent green.	Black.	Iridescent blue.	Black and yellow.	Black and rufous.	Black and reddish rufous.
Heliconius . .	42	7	3		1	16	26	10	1	5
Eueides . . .	15			2		1				
Total	57	7	3	2	1	17	26	10	1	5

TABLE 25.

Showing the Variations of the "Middle Black Stripe" of the *hind wing* in Heliconius and Eueides.

	Well developed as a more or less distinct stripe.	Absent (suffused with black).	Absent (place taken by red or rufous).	Absent (place taken by ocher color).
Heliconius	47	59	5	
Eueides	6		10	1
Total	53	59	15	1

TABLE 26.

Showing the Color-Variations of the "Outer Rufous" Area of the *hind wing* in Heliconius and Eueides.

	Rufous.	Reddish rufous.	Yellow.	White.	Ocher.	Black.	Iridescent blue.
Heliconius . .	30	4	19	3		49	6
Eueides . . .	17					1	
Total	47	4	19	3		50	6

TABLE 27.

The "Outer Black" Area of the *hind wing* is Black in 106 species of Heliconius, White in 4, and Yellow in 1. It is Black in all the 18 species of Eueides known to me.

TABLE 28.

Showing the Number of Species in each Genus of the Heliconidae examined, and also the Number known according to the Enumeration of Staudinger ('84–'88).

Genera.	Number of species examined by me.	Number of species known to Staudinger ('84–'88).
Lycorea	4 species and 1 var.	4 species and 1 var.
Ituna	3	4
Athesis	3	4
Thyridia	5	4
Athyrtis	2	2
Olyras	4	5
Eutresis	2	2
Aprotopos	2	4
Dircenna	12	20+
Callithomia	3	8
Epithomia	2	2
Ceratinia	41	50+
Sais	5	4
Scada	7	9
Mechanitis	10 species, 14 var.	10 species, 13 var.
Napeogenes	30	30+
Ithomia	212	230+
Aeria	4	4
Melinaea	24	25
Tithorea	10	18
Total of Danaoid Heliconidae.	400	453+
Heliconius	111	130
Eueides	18	24
Total	529	607+

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PLATE I.

ABBREVIATIONS.

B.	Back surface covered with wings.	O.	Orifice for admission of light.
F.	Front surface covered with wings.	S.	Spectroscope.

Arrow indicates directions of rays of light.

Fig. 1. Perspective view of spectroscopic apparatus used in determining the composition of the colors of Lepidoptera.

Fig. 2. Horizontal section of same. See p. 175.

Fig. 3. Pendulum used in determination of the frictional resistance between the air and the wings of Lepidoptera. See p. 193.

Figs. 4, 5. Diagrams to illustrate Keeler's method of projection, as applied to Lepidoptera. See p. 207.

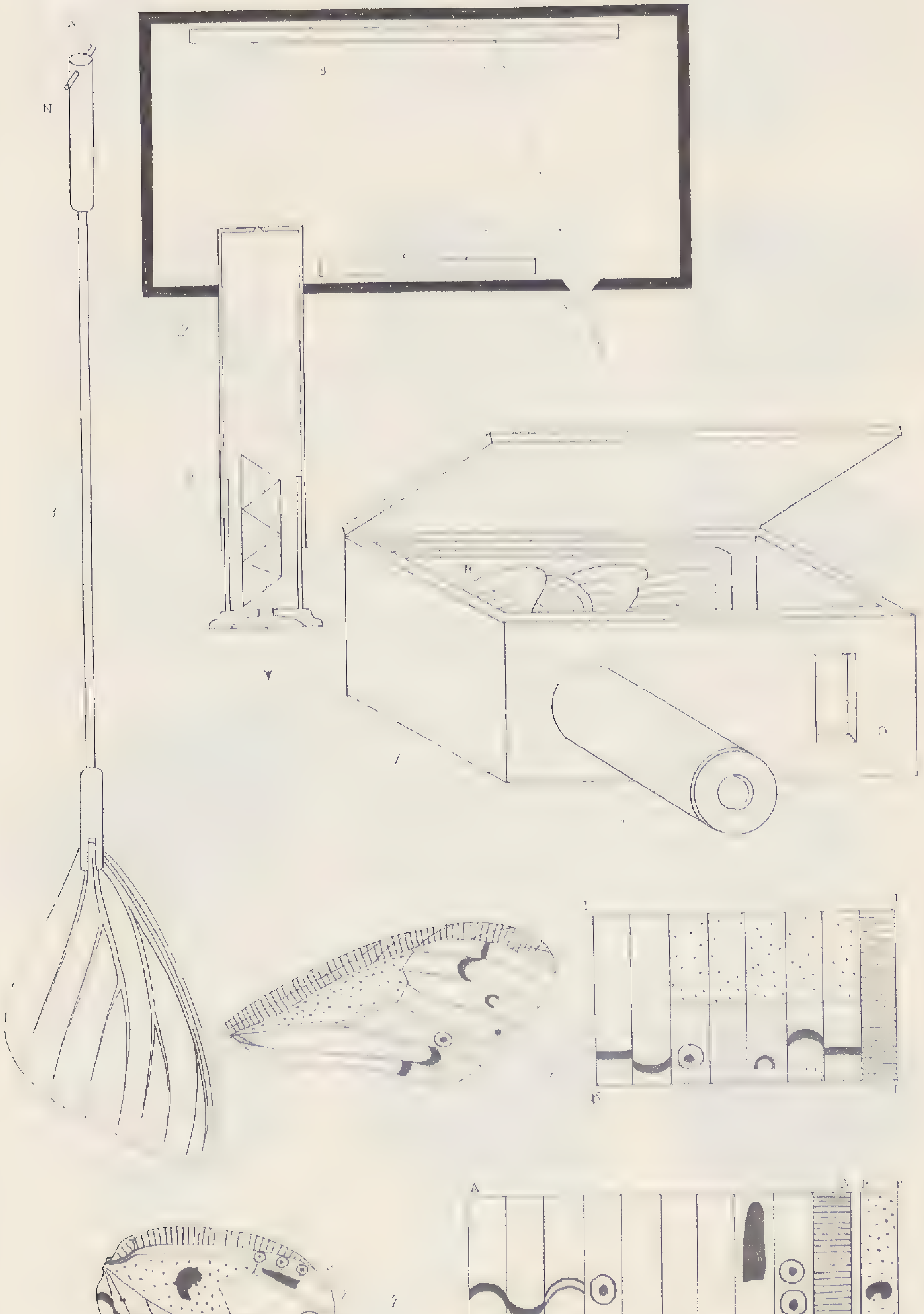
N
N
B
B
A
1
1ᵃ
1ᵇ
1ᶜ
2
3
4
5
6
7

PLATE 2.

Diagrams to illustrate the laws which govern the Color-Patterns of Lepidoptera.

Fig. 6. Euthalia bellata (W. L. Distant, '82–'86, Plate 43, Fig. 12). Illustrates the law of bilaterality of spots. See p. 183.

Fig. 7. Zethera musa (G. Semper, '86–'92, Taf. 7, Fig. 10). Bilaterality of double spots. See p. 183.

Fig. 8. Eye-spots in Morpho. See p. 182, 183.

Fig. 9. Parthenos gambrisius (W. L. Distant, '82–'86, Plate 11, Fig. 7). A series of complex spots, each one being similar to the rest, and bilaterally symmetrical.

Figs. 10, 11. Ornithoptera urvillana and O. priamus (R. H. F. Ripon, '89–'93). Spots within spots, all being bilaterally symmetrical.

Figs. 12, 13. Hestia jasonia and H. leuconoe. Axis of lateral symmetry (H, H), for spots passes through center of interspace. H. jasonia (F. Moore, '90–'96, Plate 3, Fig. 1). H. leuconoe (G. Semper, '86–'92, Taf. 1, Fig. 3).

Fig. 14. Papilio emalthion, to illustrate fusion of two rows of spots.

Fig. 15. Ornithoptera trojana, an apparent exception to the law of bilaterality. See p. 187.

Fig. 16. Limenitis proserpina (S. H. Scudder, '88–'89, Plate 2, Fig. 9), showing fusion of two rows of differently colored spots. See p. 187.

Fig. 17. Saturnia spini, false eye-spot. See p. 187.

Fig. 18. Cases of degeneration of bands of color. See p. 184.

Fig. 19. Missanga patina (F. Moore, '90–'96, Plate 72, Fig. 2^c). Exceptional form of eye-spot. See foot note p. 186.

Figs. 20–23. Hypothetical conditions of coloration, not found in nature, being contrary to the laws of color-pattern. See p. 188.

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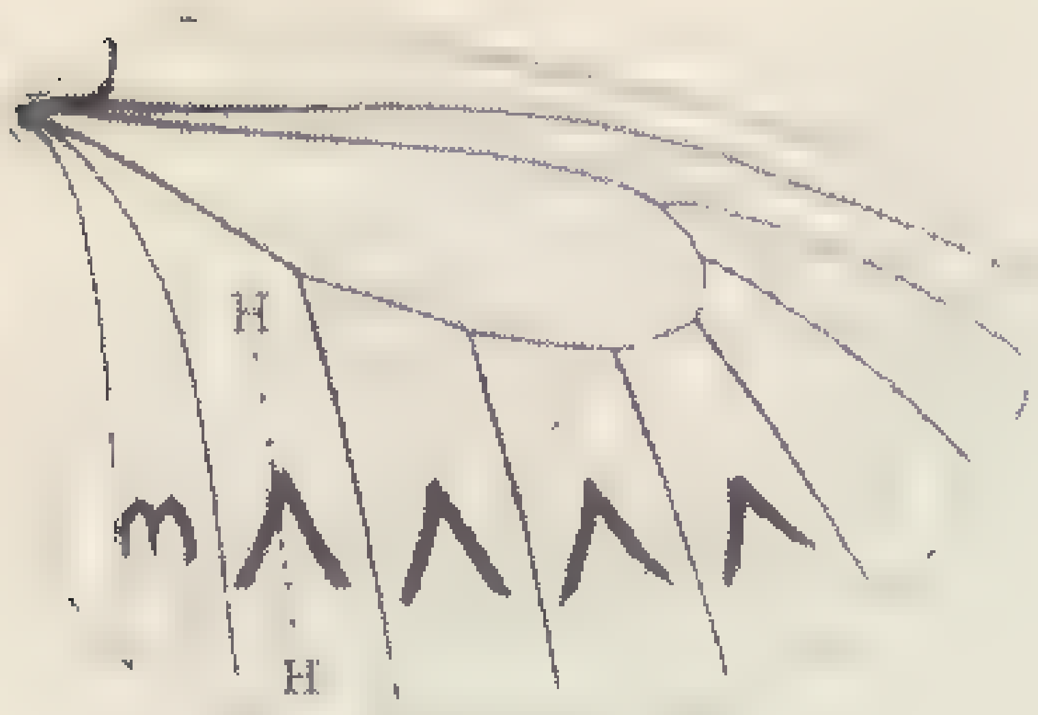

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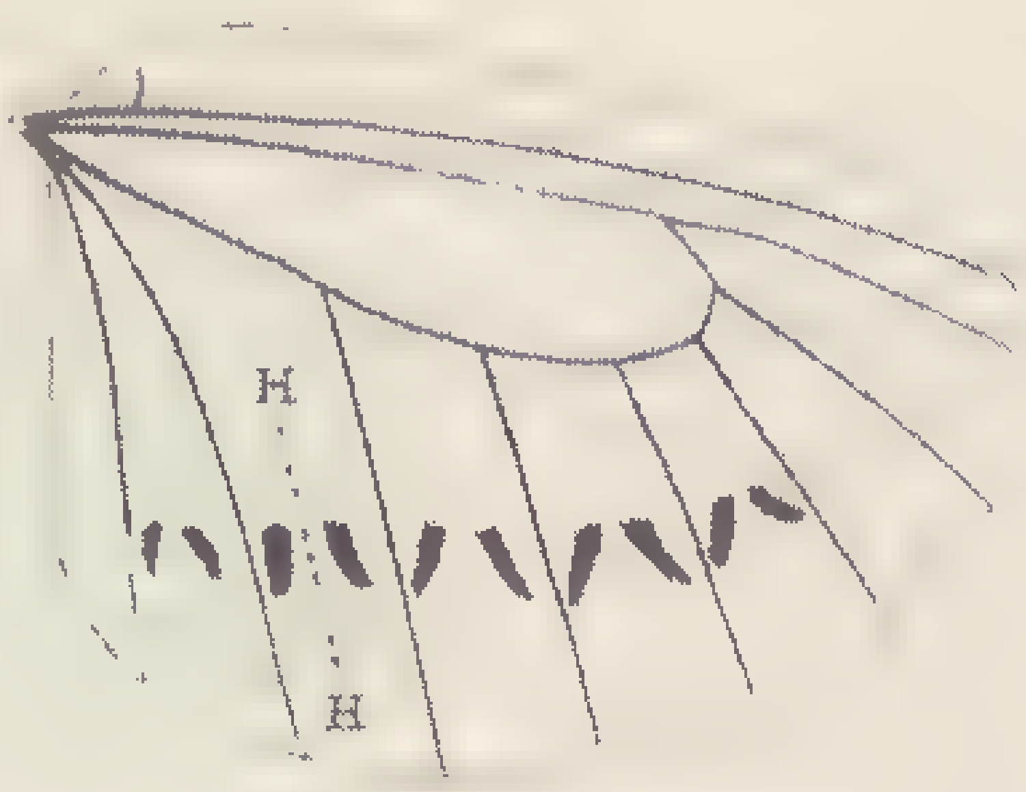

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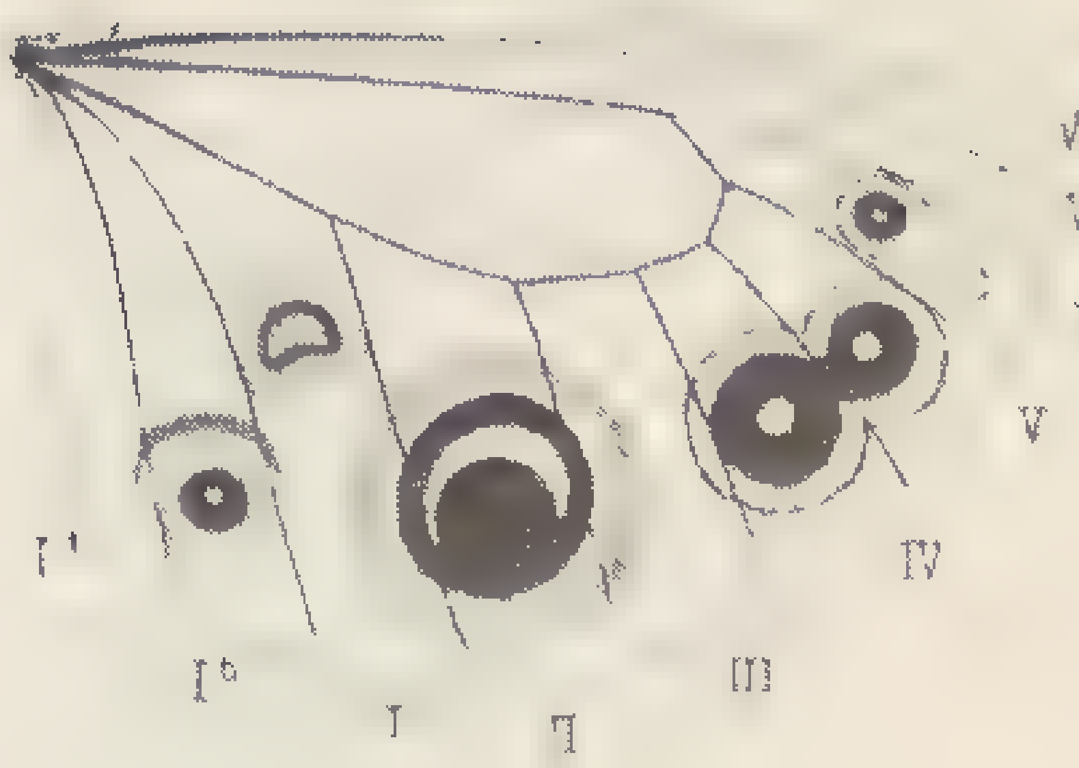
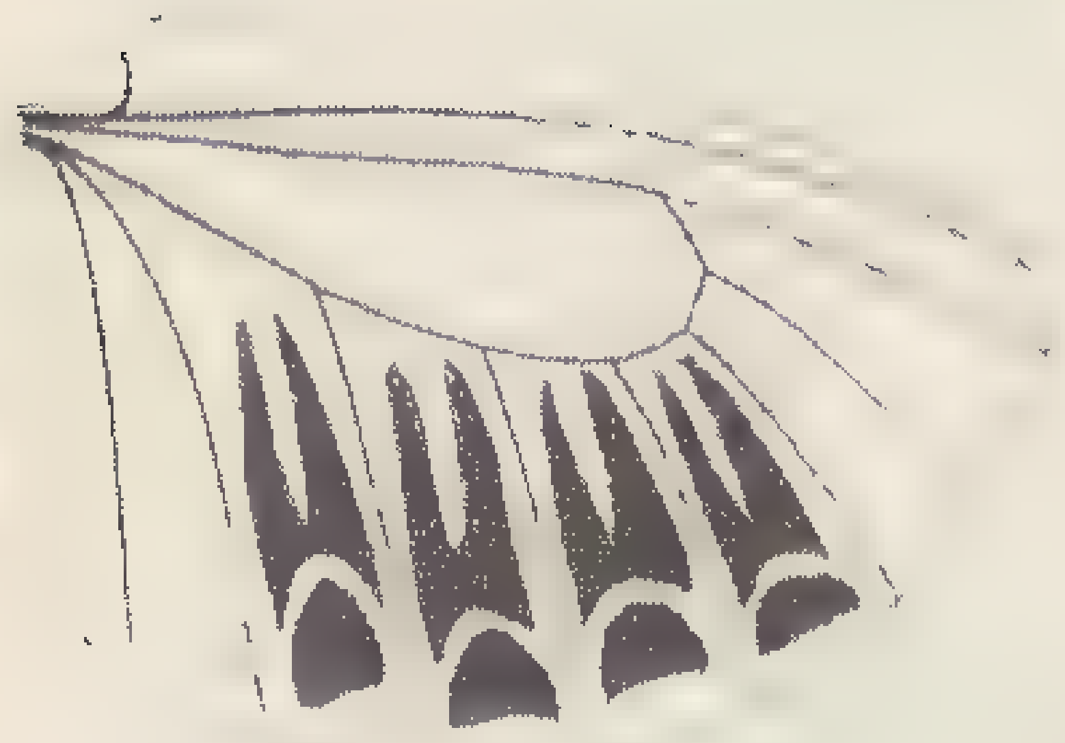

VII
VI
VI
V
IV
III
II
I
I°
I'

9


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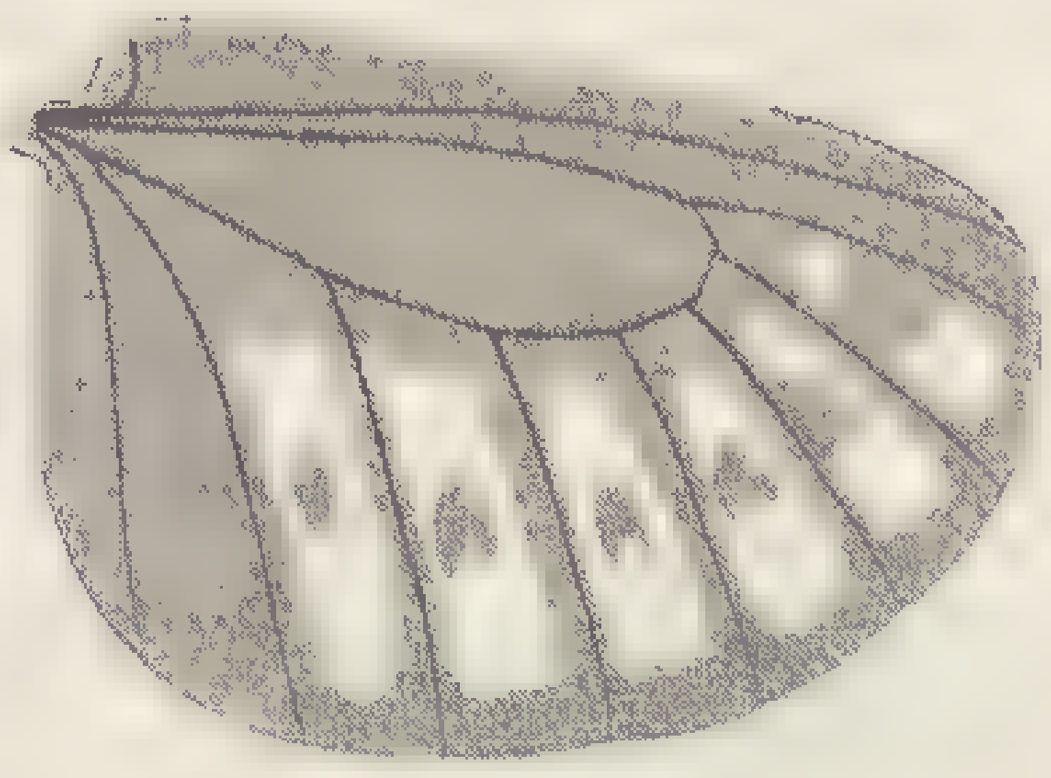

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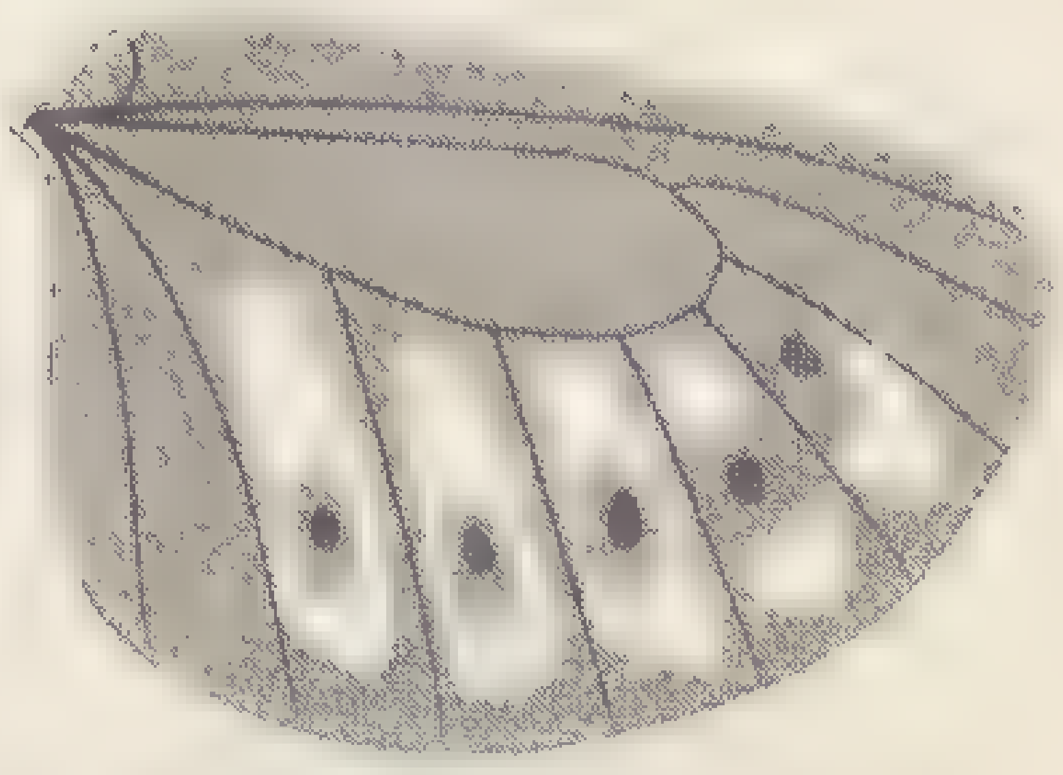

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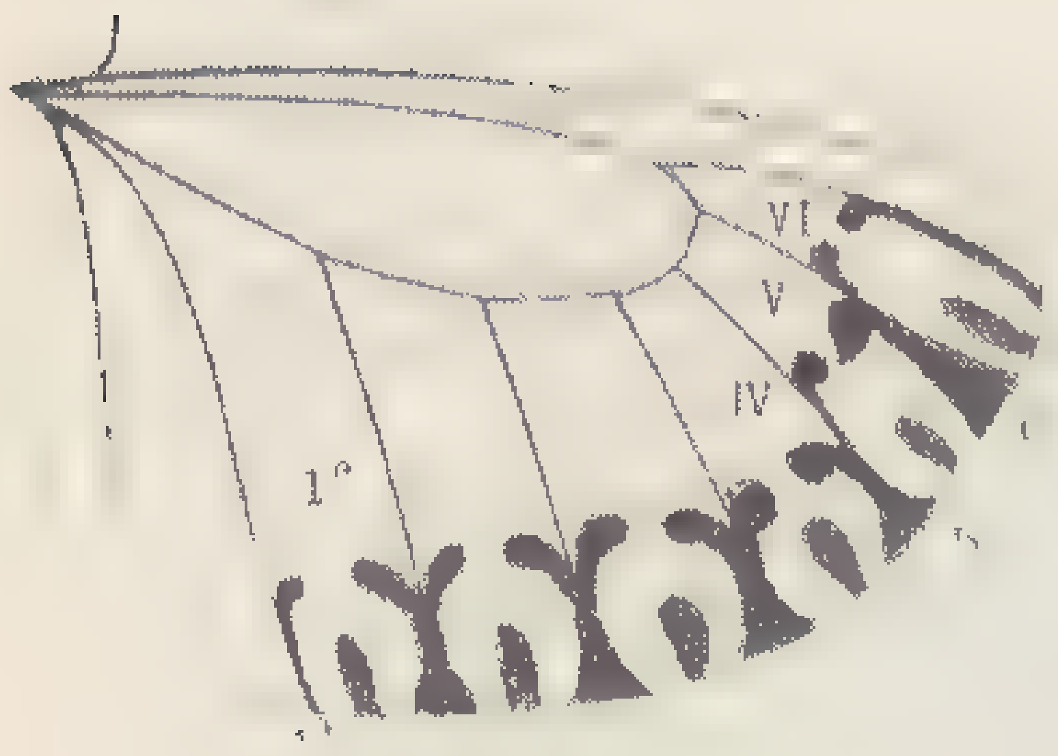

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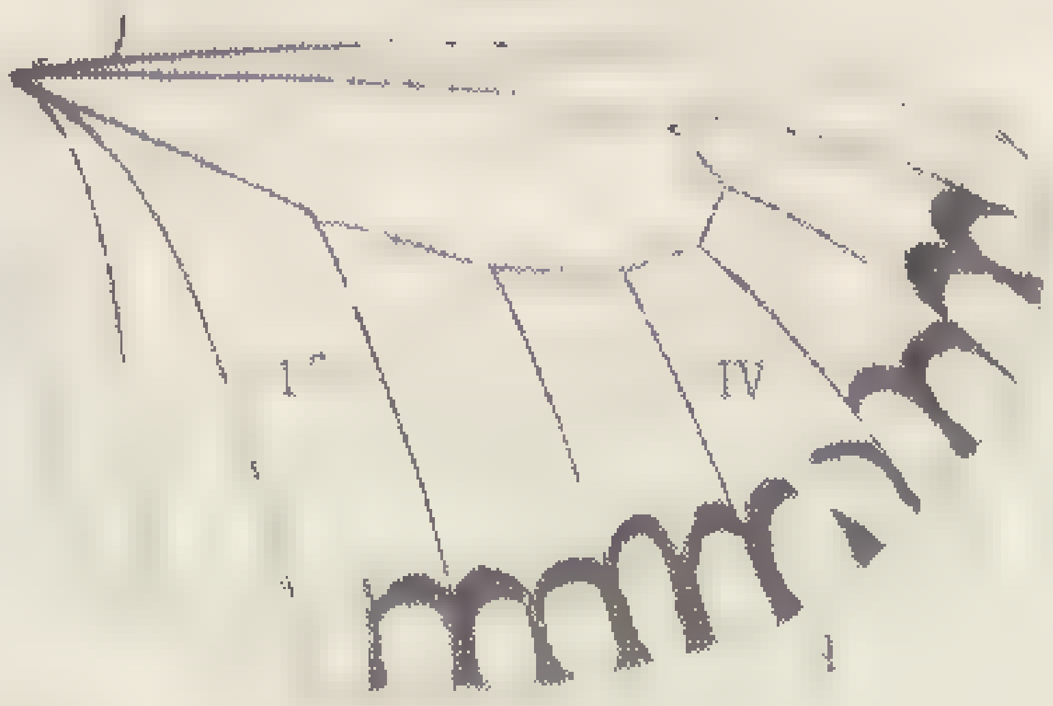

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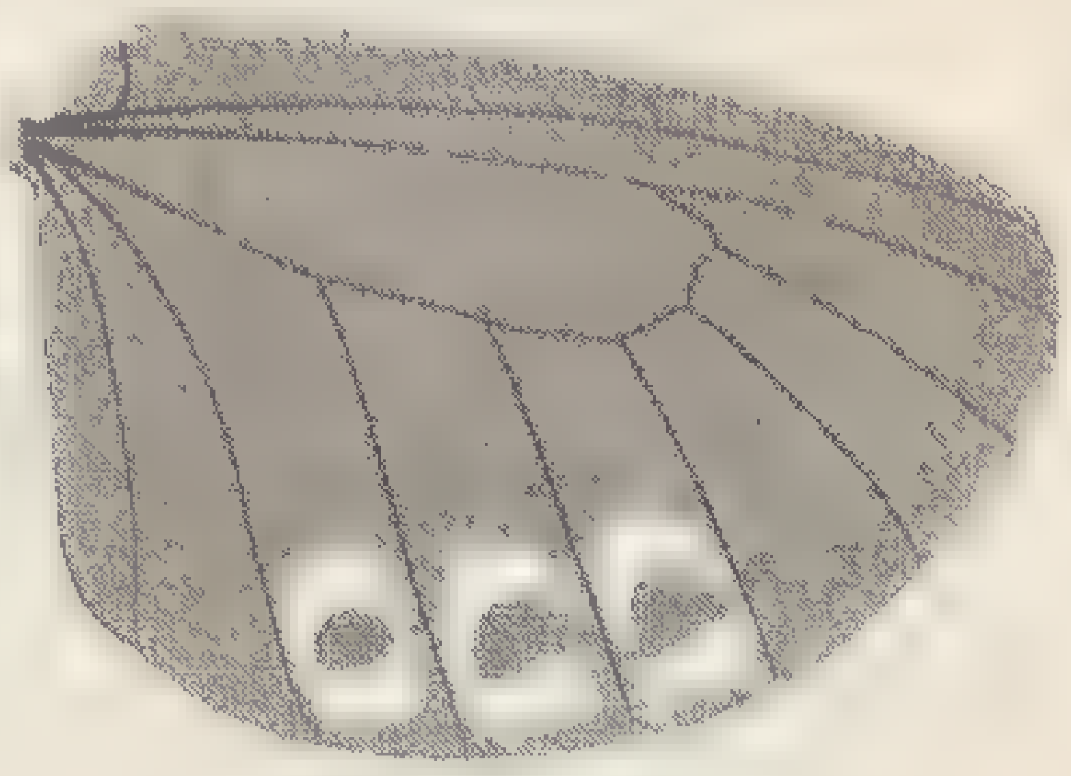

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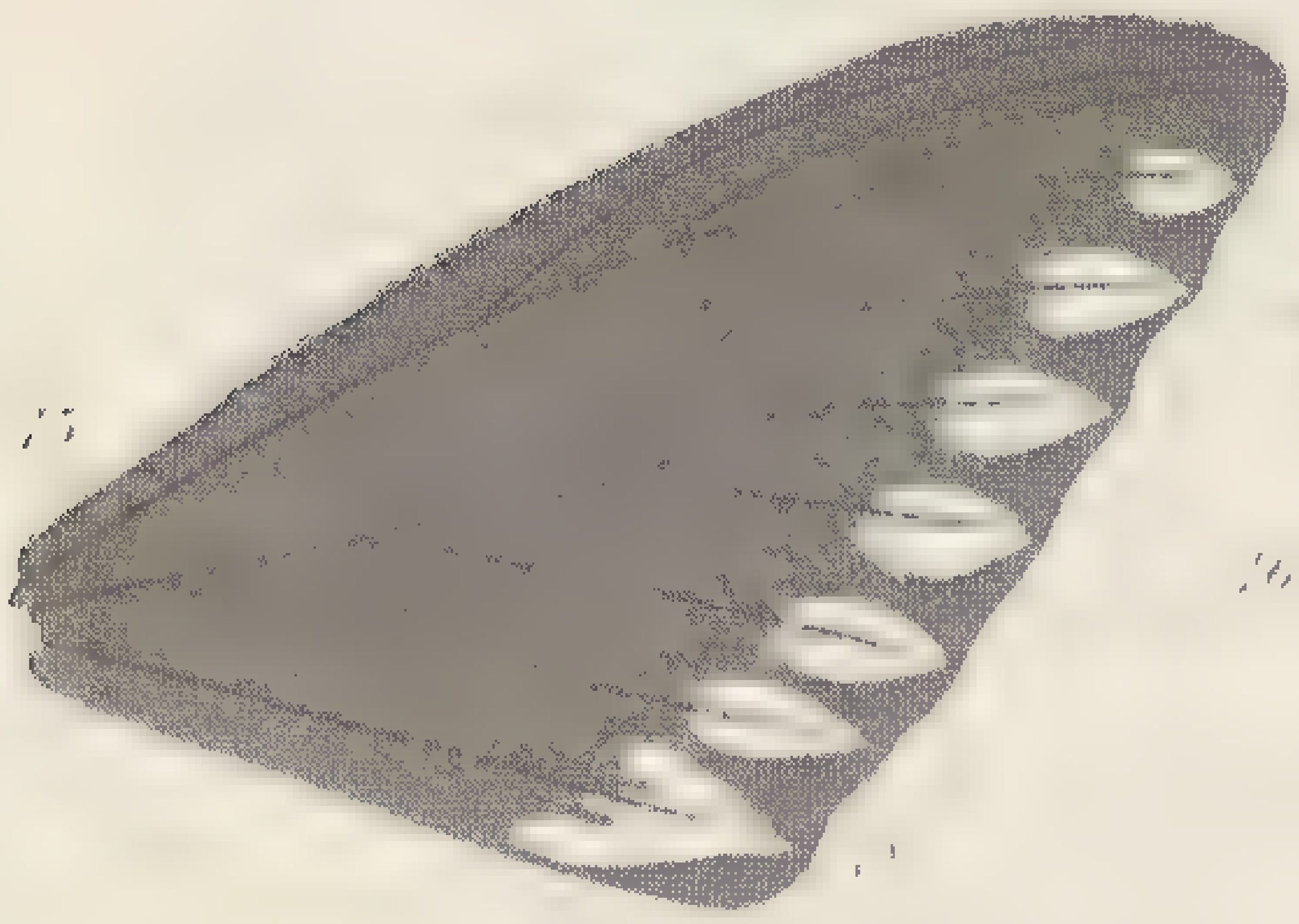

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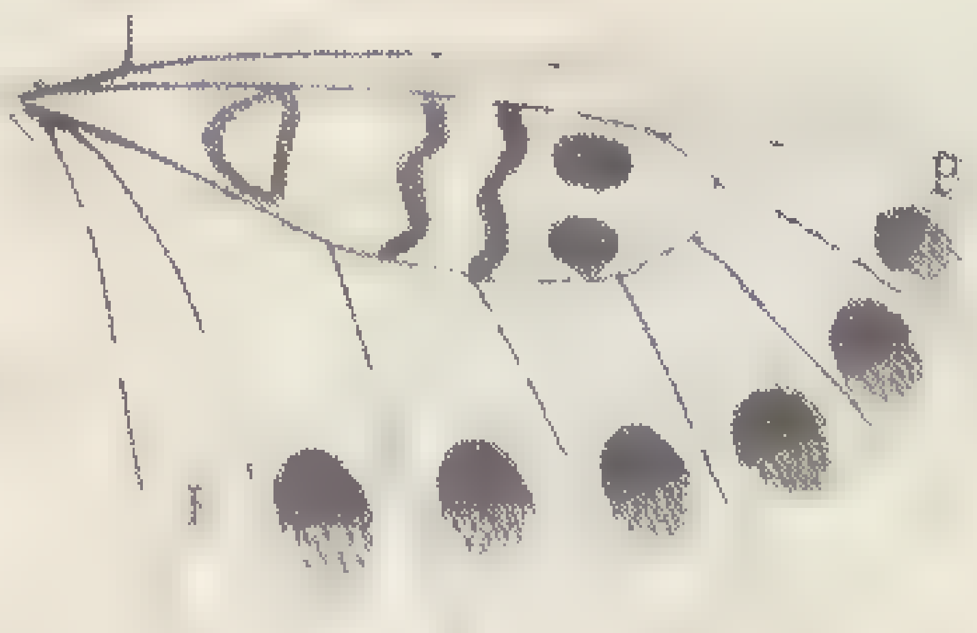

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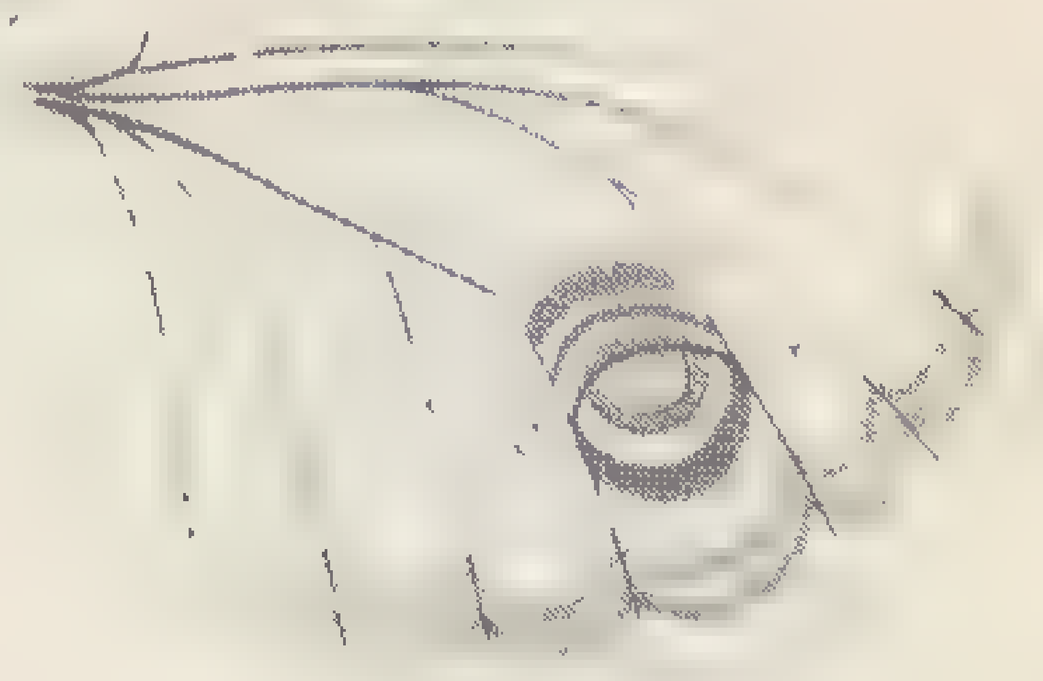

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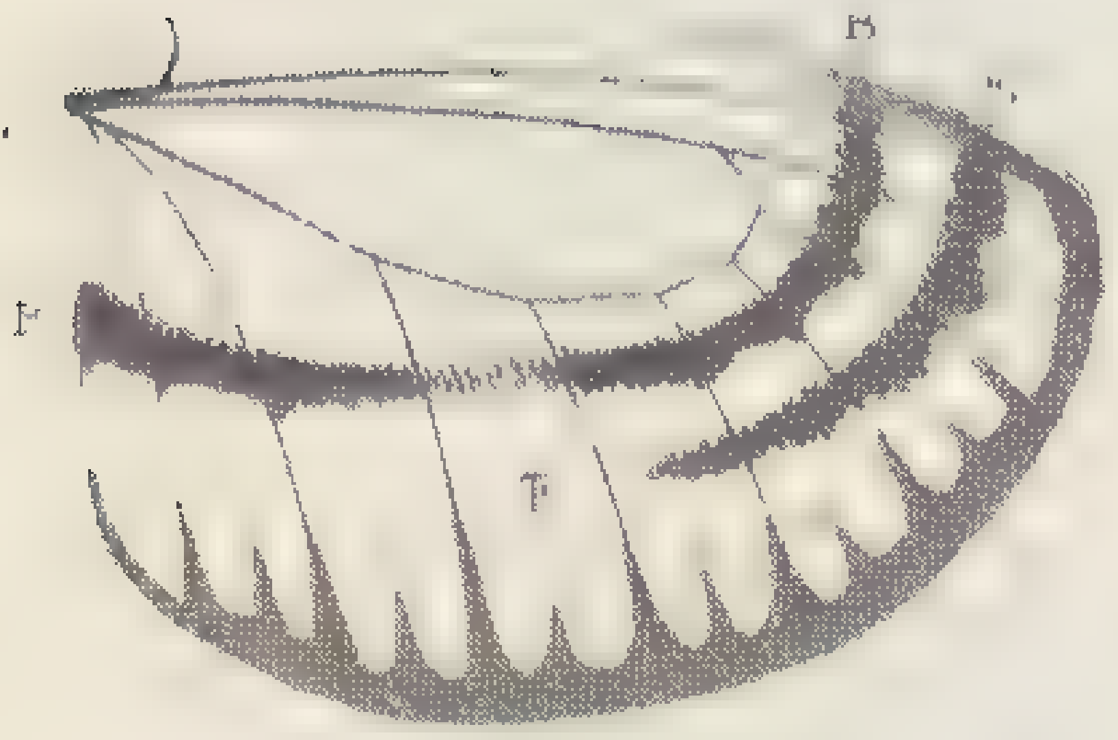

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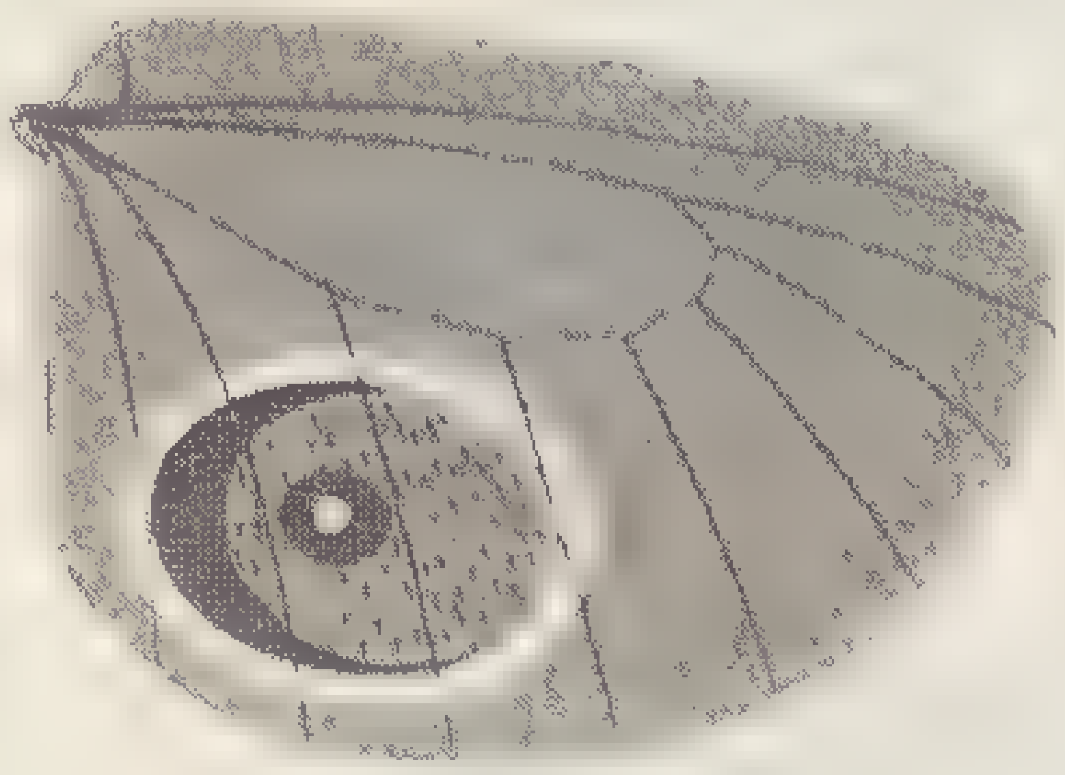

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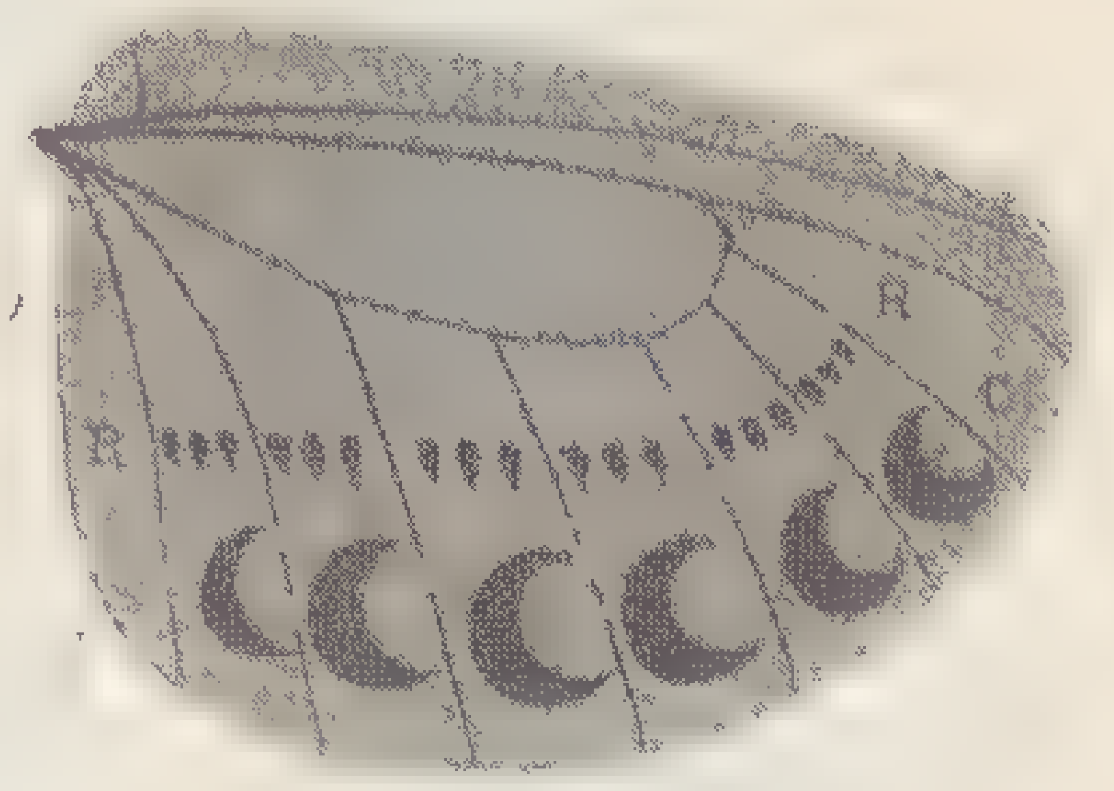

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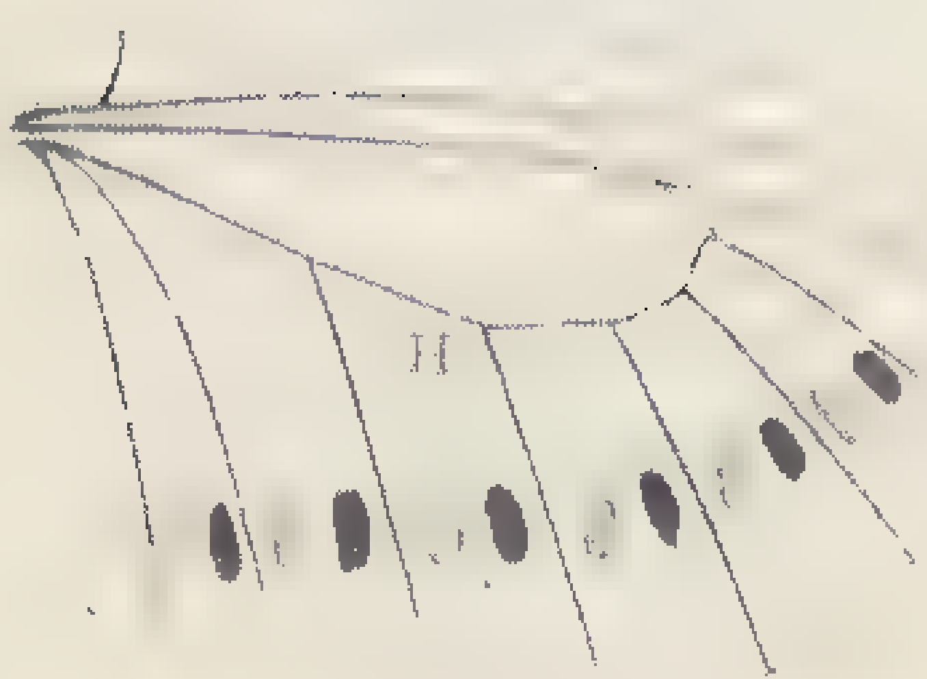

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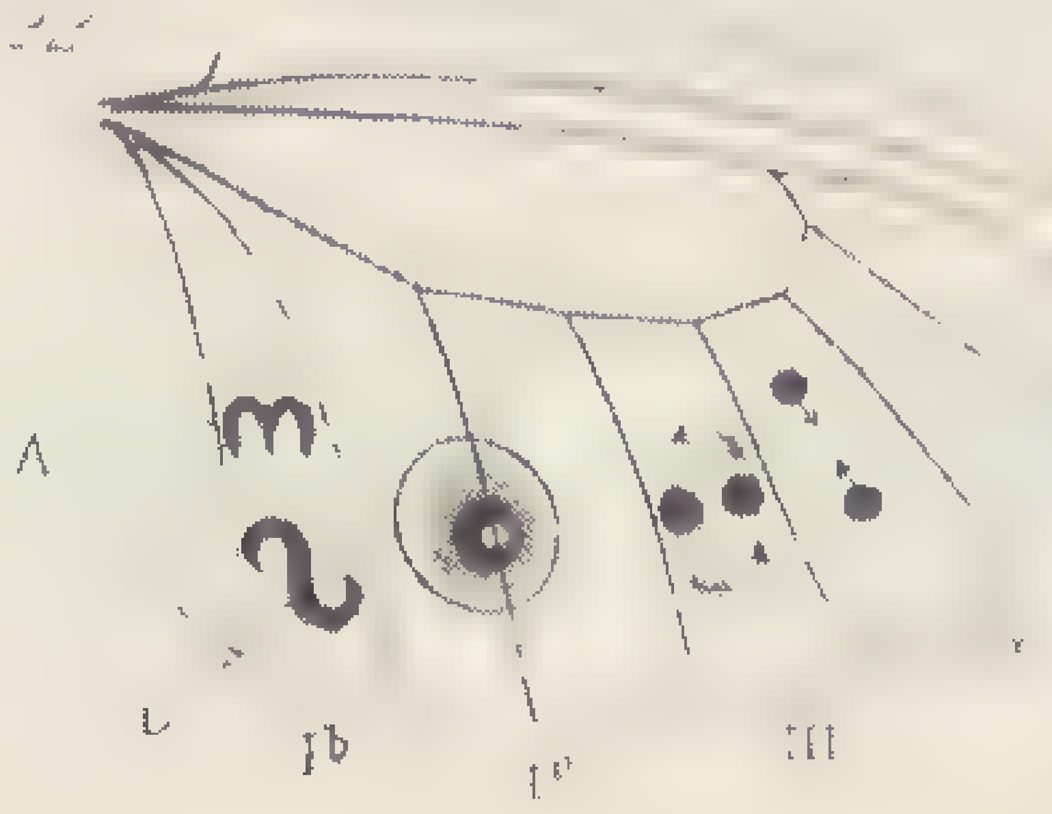

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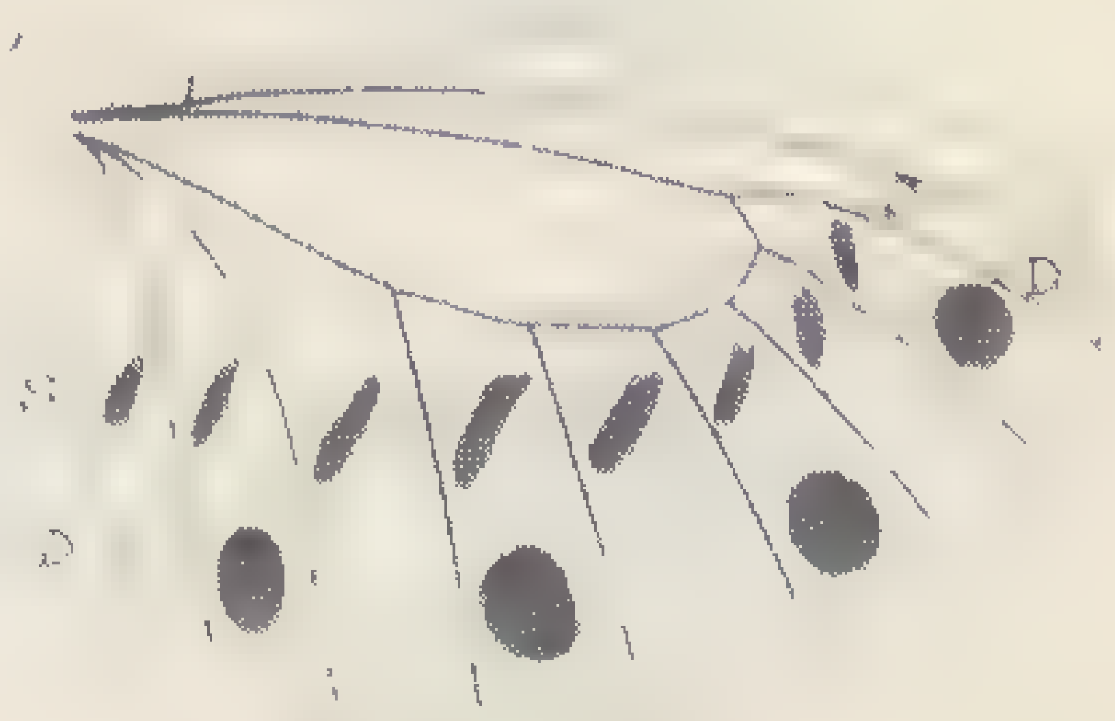


B Meisel lith.

PLATE 3.

To illustrate color-development in Callosamia promethea and Danais plexippus.

Fig. 24. Enlarged view of pupal wing of C. promethea in the "white stage." See p. 178.

Fig. 25. Scale from wing of C. promethea in white stage of color-development, showing the total absence of pigment in the scale. See p. 178.

Fig. 26. Scale from light drab-colored area of mature wing of C. promethea.

Figs. 27, 36, and 33. Successive stages in the formation of color in pupal hind wing of C. promethea.

Figs. 28, 37–40. Successive stages in the formation of color in the pupal fore wing of ♀ C. promethea. See p. 179, 180.

Figs. 29, 30–35. Successive stages in the formation of color in the pupal wings of ♂ C. promethea. (Figs. 29, 30–33, 35 fore wing; Fig. 34 hind wing.) See p. 179, 180.

Figs. 34 and 41. Pupal hind wings of C. promethea, respectively mature ♂ and ♀.

Figs. 42–45. Successive stages in the color-development of D. plexippus. See p. 180–181.

[illegible] Plate 3

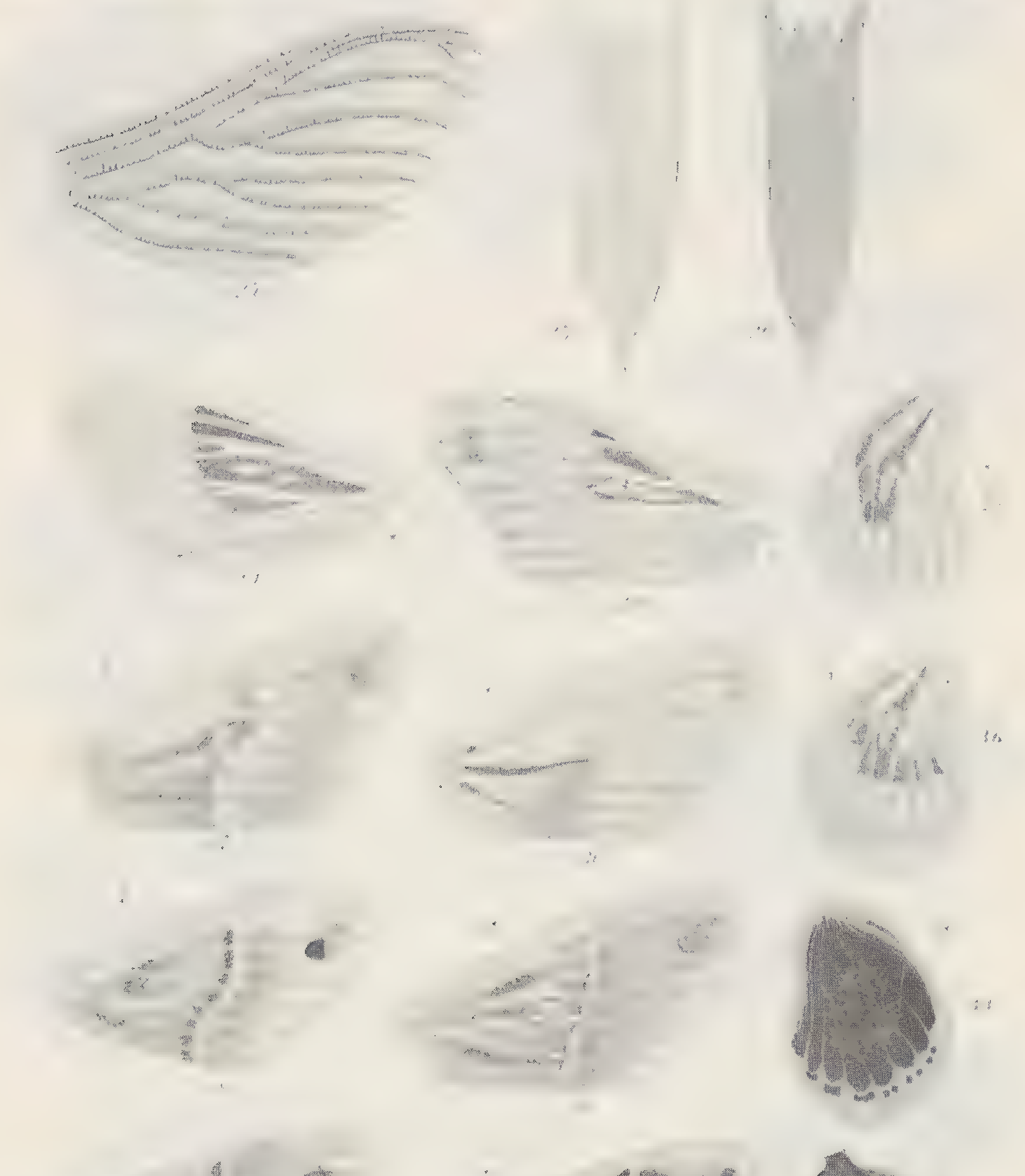
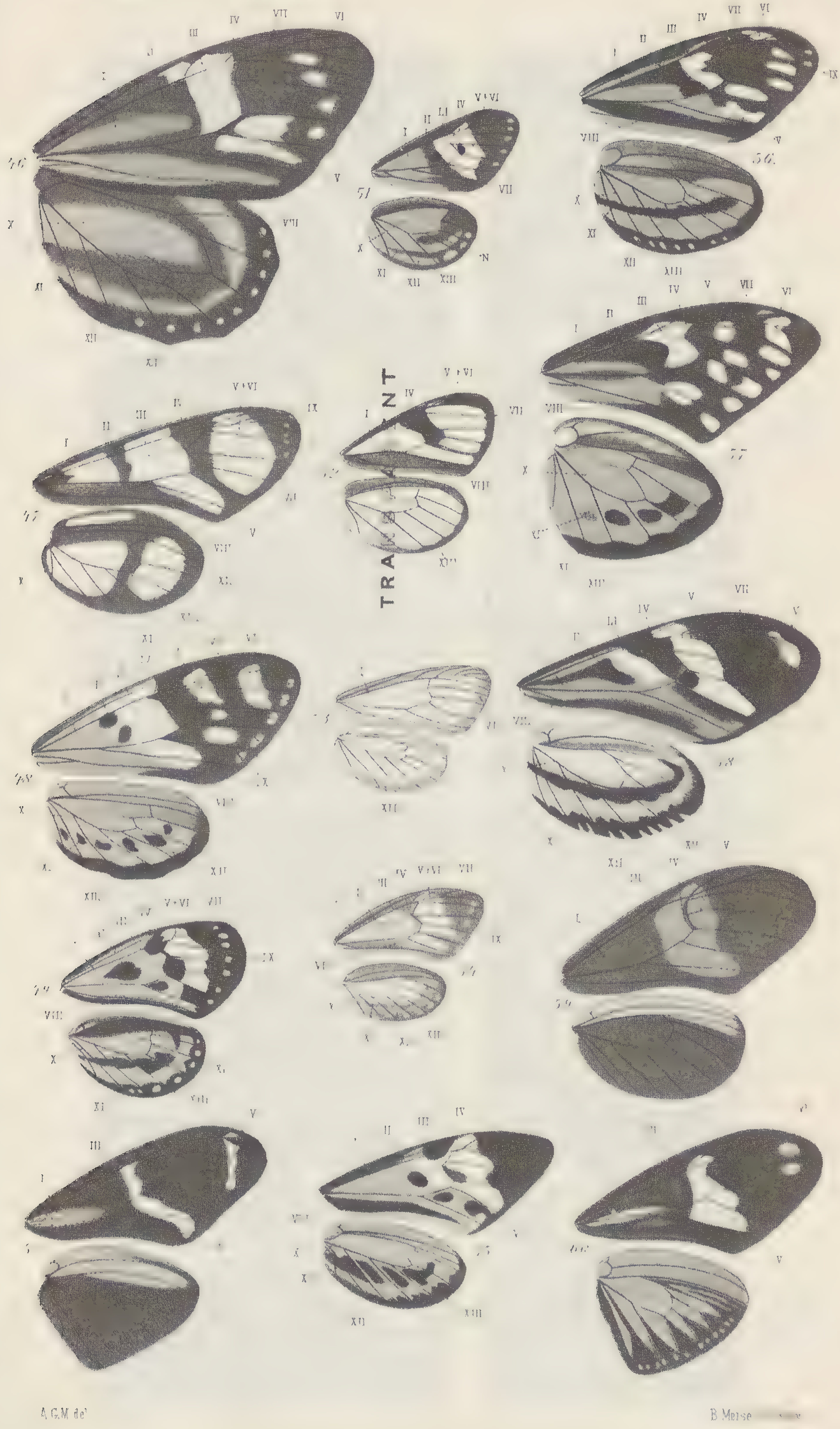


PLATE 4.

Systematic analysis of the characteristic markings upon the wings of the Heliconidae. Homologous markings are designated by the same numerals, I, II, III, etc.

Fig. 46.	Lycorea ceres; an example of the "Melinaea type" of coloration. See p. 205.
Fig. 47.	Thyridia psidii; an example of the "Ithomia type" of coloration. See p. 206 and Plate **7**, Fig. 79.
Fig. 48.	Melinaea paraiya.
Fig. 49.	Ceratinia ninonia.
Fig. 50.	Heliconius antiochus.
Fig. 51.	Napeogenes duessa.
Fig. 52.	Ithomia sao.
Fig. 53.	Melinaea gazoria.
Fig. 54.	Ithomia nise.
Fig. 55.	Mechanitis polymnia.
Fig. 56.	Eueides cleobaea.
Fig. 57.	Tithorea furia var.
Fig. 58.	Heliconius eucrate.
Fig. 59.	Heliconius melpomene.
Fig. 60.	Heliconius erato.


46
51
56
47
TRANSPARENT
77

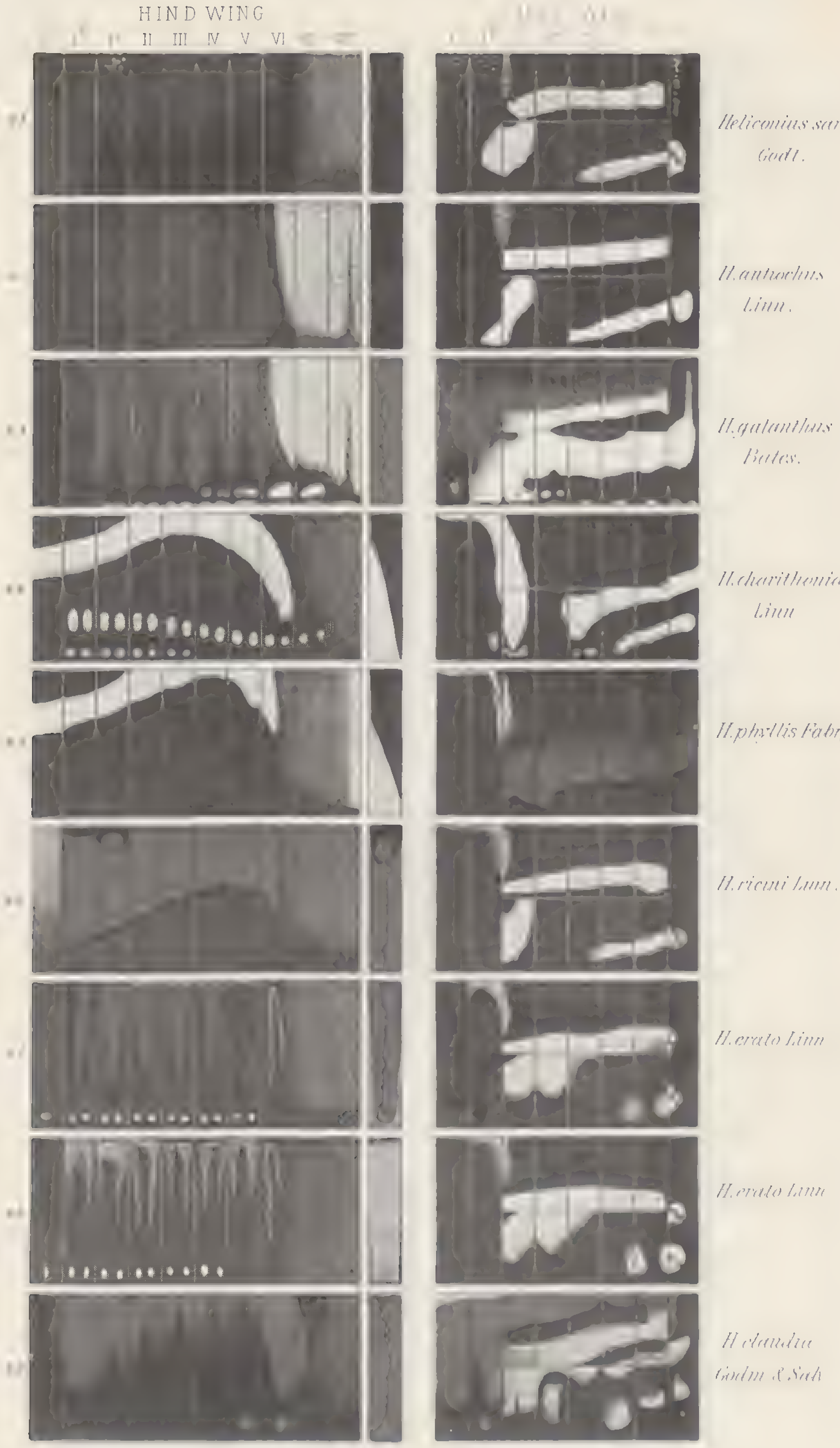
A. G. M. del.

B. Meisel lith.

PLATE 5.

Color-patterns of the Antiochus and Erato groups of the Heliconidae projected by Keeler's method. See p. 207.

Figs. 61, 62. Heliconius sara and H. antiochus, to show variation of yellow to white. See p. 210, and Fig. 50, Plate **4**.
Fig. 63. H. galanthus, showing development of white.
Fig. 64. H. charitonia, rows of double spots.
Fig. 65. H. phyllis, close relation between yellow and red.
Fig. 66. H. ricini.
Figs. 67, 68. H. erato; two color-types.
Fig. 69. H. claudia; an example of the Sylvanus group.


HIND WING
II
III
IV
V
VI
Heliconius sara Godt.
H. antiochus Linn.
H. galanthus Bates.
H. charithonia Linn
H. phyllis Fabr.
H. ricini Linn.
H. erato Linn
H. erato Linn
H. claudia Godm. & Salv.

A.G.M. de.

B. Me[illegible] lith. Bo[illegible]

PLATE 6.

Color-patterns of the Melpomene group of Heliconius and of the genus Eueides.

Fig. 70. H. melpomene, the type of the Melpomene group. See p. 212, and Fig. 59, Plate **4**.

Fig. 71. H. melpomene var. callicopis, showing the breaking up of the red area of the primaries.

Fig. 72. H. melpomene var. cybele; the fore wing has assumed a color-pattern which recalls the "Melinaea type" of coloration found in the Danaoid Heliconidae.

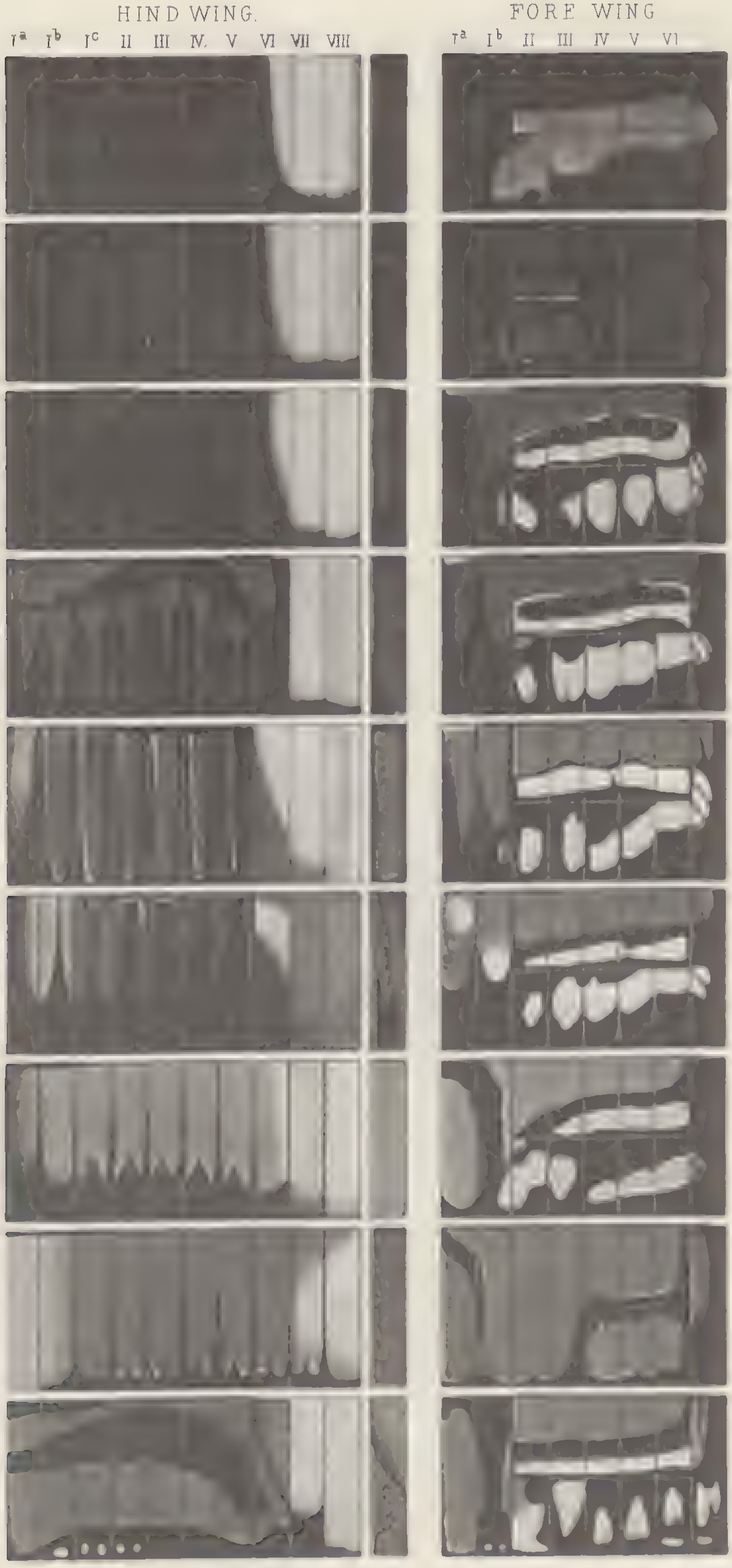
Fig. 73. H. thelxiope, derived phylogenetically from H. melpomene, and showing a rather close approach to the "Melinaea type" of coloration. See p. 212.

Fig. 74. H. vesta.

Fig. 75. Eueides thales ♂; represented to show the close resemblance of its color-pattern to H. vesta. See p. 212.

Figs. 76, 77. E. mereaui and E. aliphera. E. mereaui is intermediate in color-pattern between E. thales and E. aliphera.

Fig. 78. Eueides cleobaea, to show the close approach of this insect to the "Melinaea type" of coloration.


HIND WING.
FORE WING
Iᵃ Iᵇ Iᶜ II III IV. V VI VII VIII
Iᵃ Iᵇ II III IV V VI

Heliconius melpomene Linn

H. melpomene var. callycopis Cram

H. melpomene var. cybele Cram.

H. Thelxiope Hübn. ♂

H. vesta Cram.

Eueides thales Cram. ♂

E. mereaui Hübn

E. aliphera Godt

E. cleobaea Hübn

PLATE 7.

Intended to show some types of coloration which are found in the Danaoid Heliconidae, and also the remarkable individual variation in Mechanitis isthmia.

Fig. 79. Thyridia psidii, an example of the "Ithomia" type of coloration. See p. 213, and Plate **4**, Fig. 47.

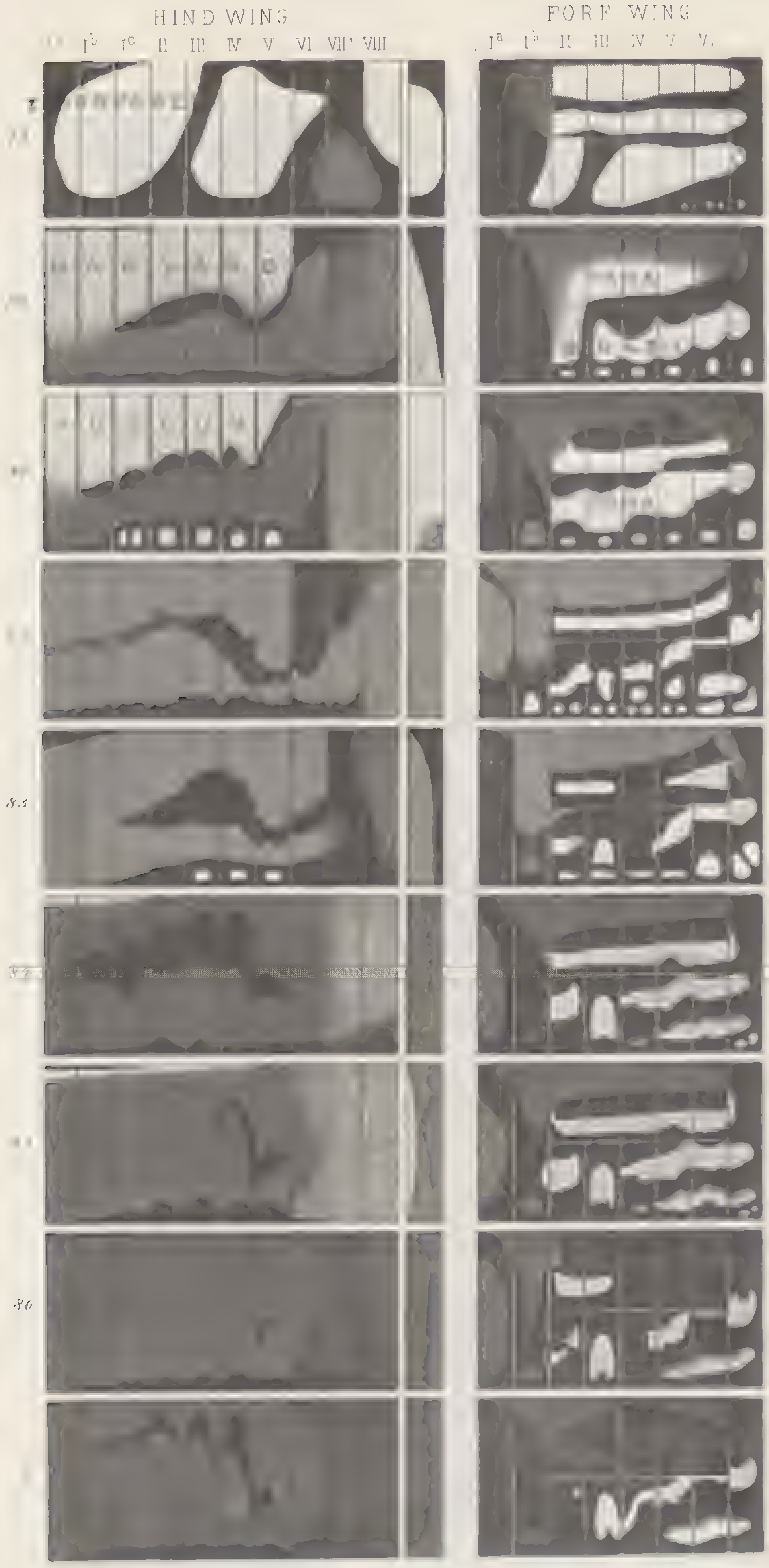
Fig. 80. Napeogenes cyrianassa, showing semi-translucent condition of wings. See p. 213.

Fig. 81. Ceratinia vallonia.

Fig. 82. Melinaea parallelis; albinism of spots on primaries; black band of hind wing broken in the middle. See p. 188, 213.

Fig. 83. Ceratinia leucania, which probably mimics M. parallelis.

Figs. 84–87. Mechanitis isthmia, showing remarkable individual variation in the black stripe of the hind wings, and also in the "inner yellow" spot of the fore wings. See p. 184, 213.


HIND WING
I^a I^b I^c II III IV V VI VII VIII
FORE WING
I^a I^b II III IV V VI
83
84
86

Thyridia psidii Linn

[illegible]

[illegible] vallonia Hew.

Melinaea parallelis Butl.

Ceratinia leucania Bates.

Mechanitis isthmia Bates.

Mechanitis [illegible]

Mechanitis isthmia Bates.

Mechanitis isthmia Bates

PLATE 8.

Illustrates the mimicry between members of the "Sylvanus" group of the genus Heliconius and various Melinaeas, etc.

Fig. 88. Heliconius eucoma, an example of the "Sylvanus" type of coloration in genus Heliconius. See p. 214.

Figs. 89, 90. Heliconius dryalus and Melinaea paraiya; close resemblance of their color-patterns. See p. 214.

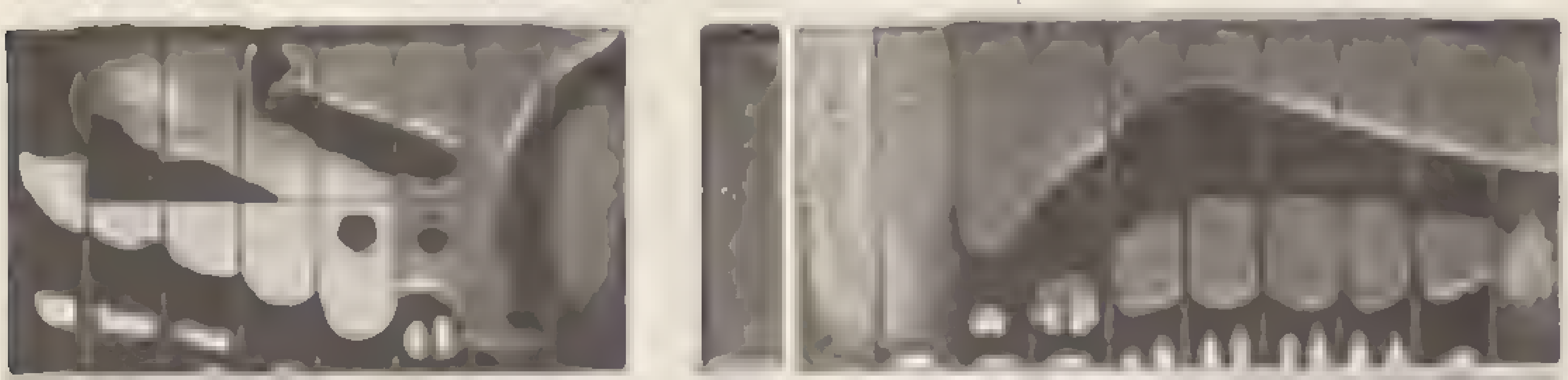
Figs. 91, 92, 93, 94. Respectively Heliconius eucrate, Melinaea thera, Eueides dianasa, and Mechanitis polymnia; showing close resemblance between color-patterns. See p. 214.

Figs. 95, 96. Heliconius sylvana and Melinaea egina; these two forms are said by Bates to mimic each other. See p. 214.

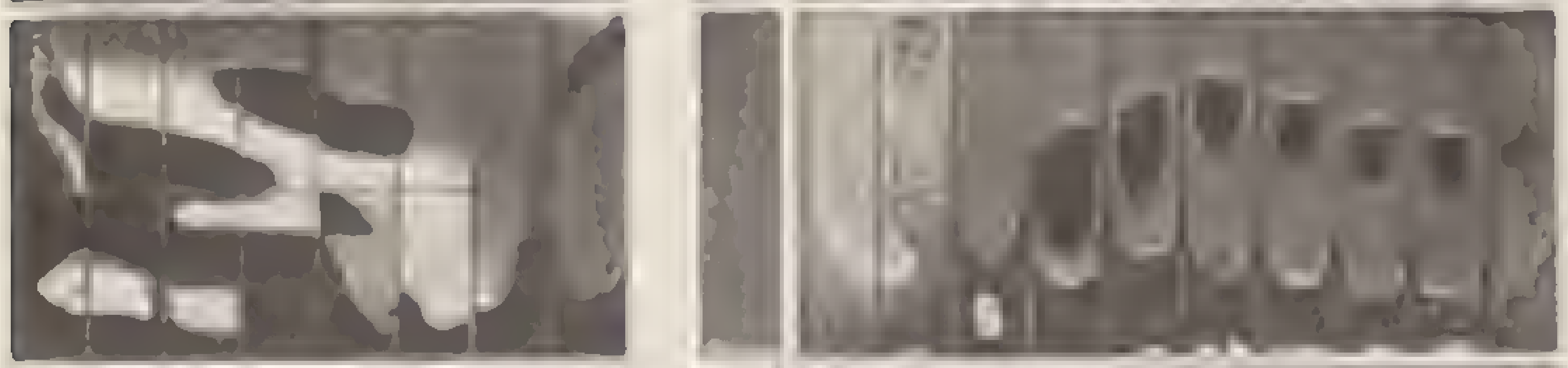
PLATE. 8.

HIND WING.

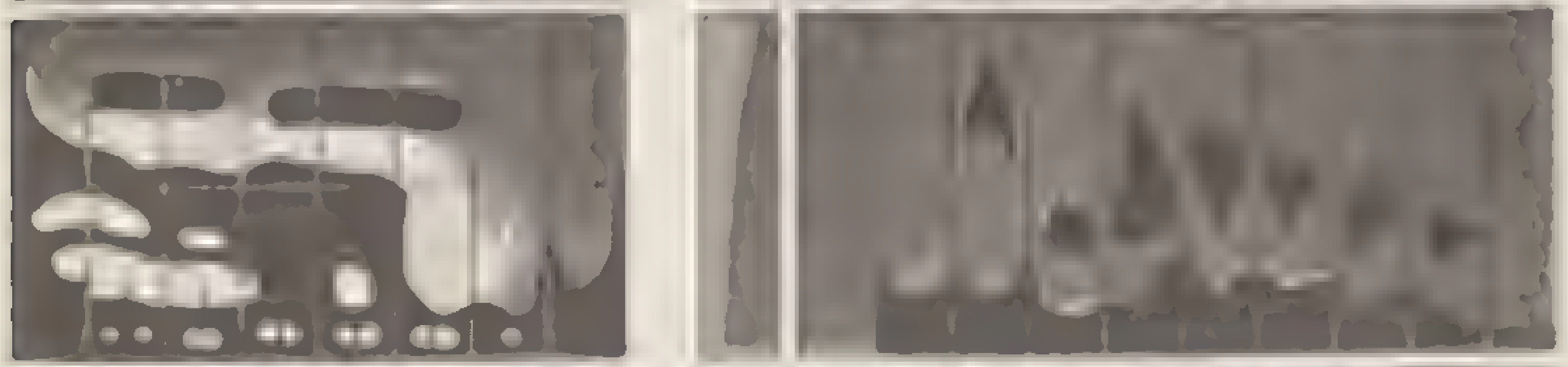
FORE WING

88


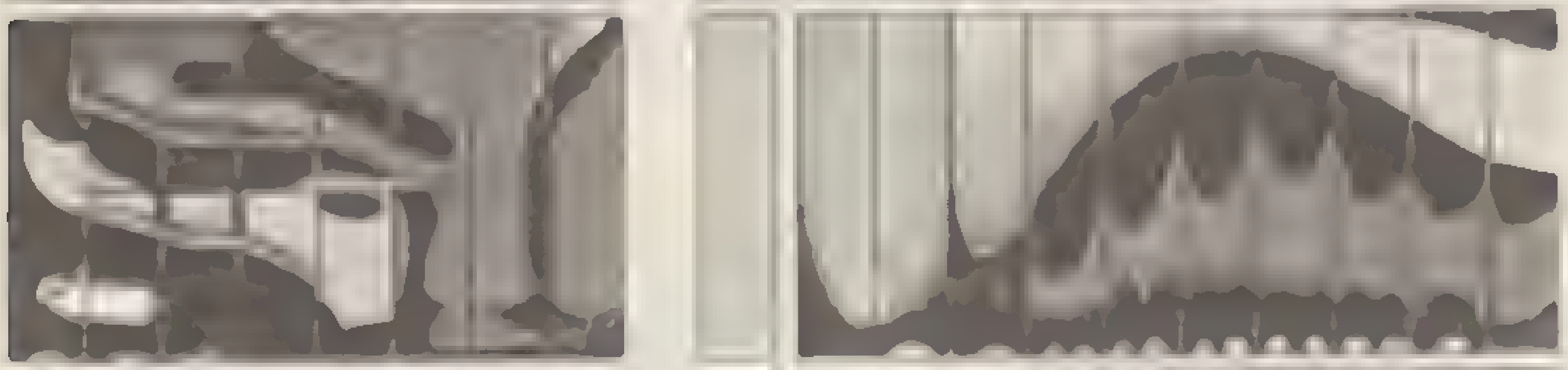
[illegible] eucoma Hübn.

89


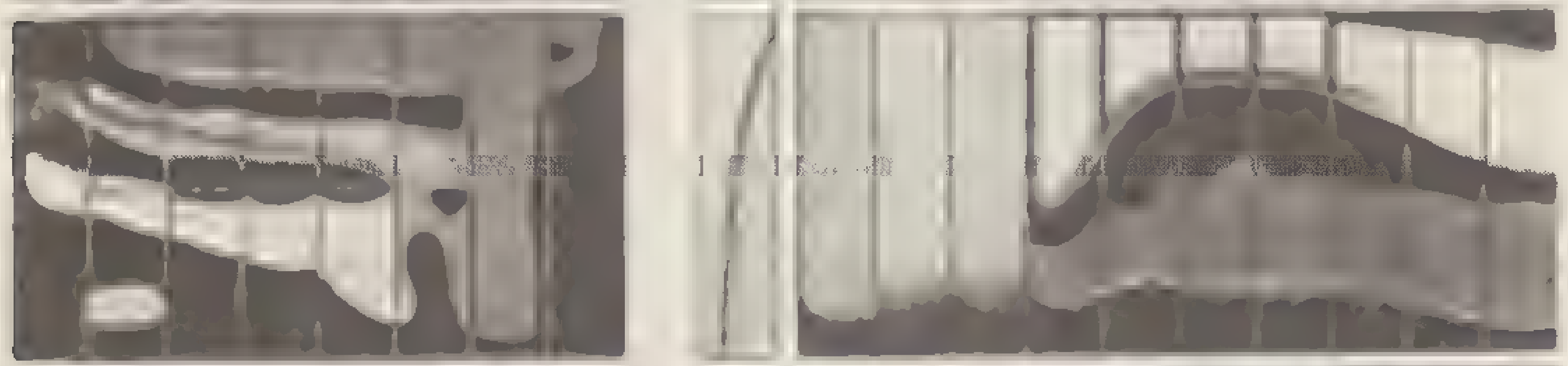
Heliconius dryalus Hopff.

90


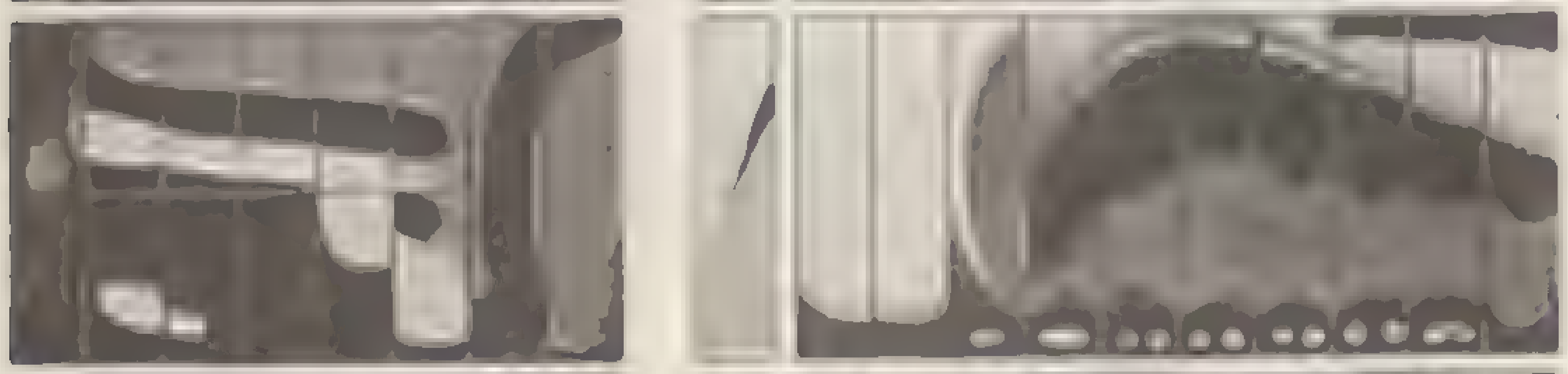
Melinaea paraiya Reak.

91.


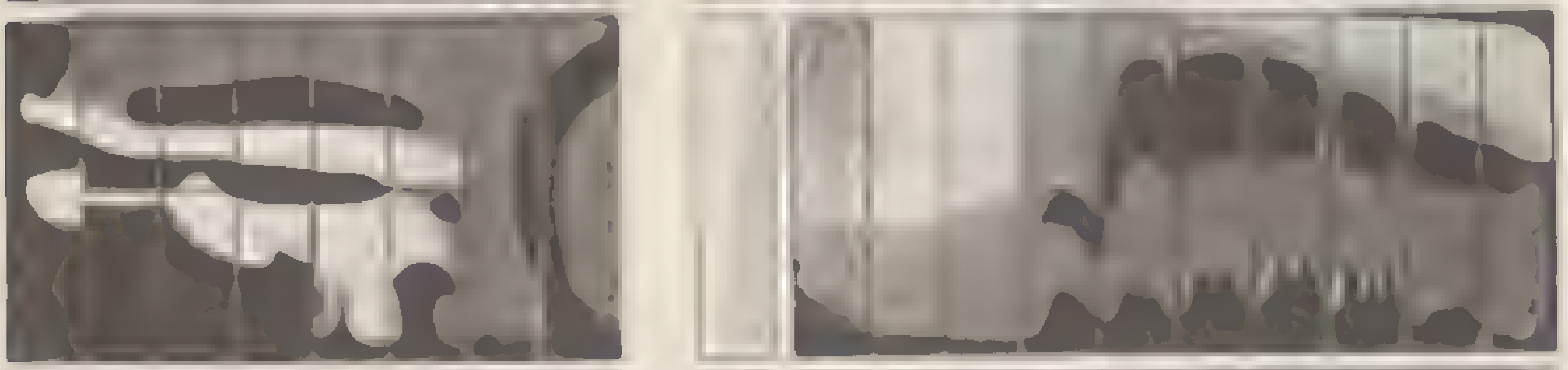
Heliconius eucrate Hubn

92


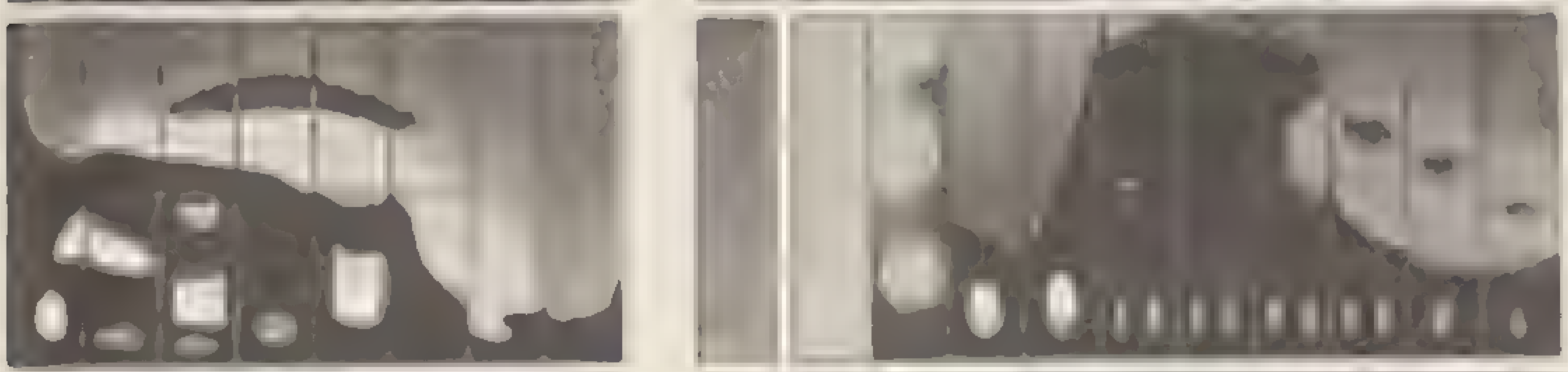
Melinaea [illegible]

93


Eueides dianasa [illegible]

94


Mechanitis [illegible]

95


Heliconius [illegible]

96

Melinaea egina Cram

PLATE 9.

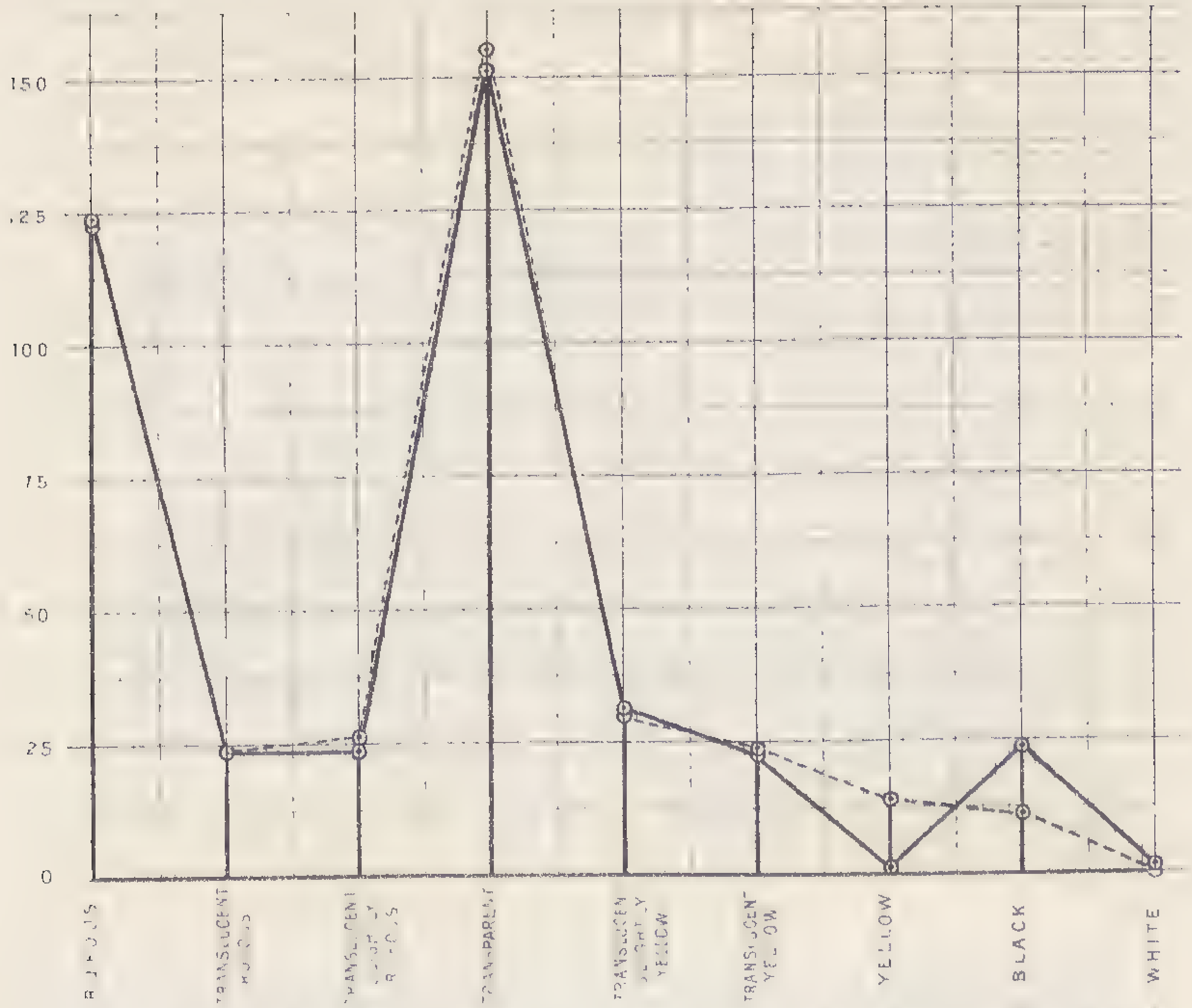
Diagrams to illustrate color-variations.

The various colors are laid off at definite intervals along the axis of abscissae, and the ordinates represent the number of species which exhibit the various colors.

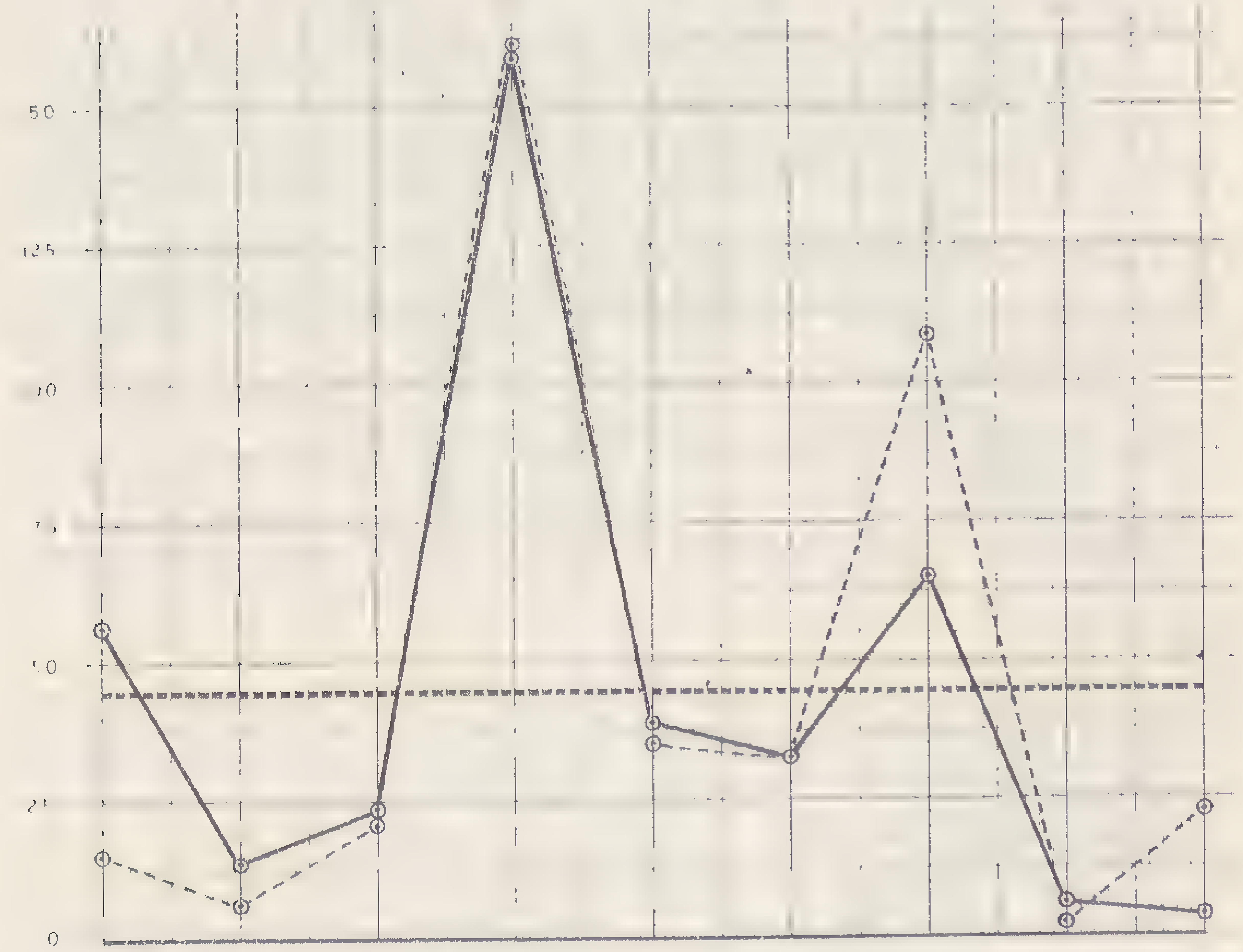
Fig. 97. Represents the color-variations of the "inner rufous" area of the fore and hind wings in the Danaoid Heliconidae. The full line represents the variations of the *fore* wing. The dotted line those of the *hind* wing. The closeness of these two lines shows the intimate relations between the color-variations of the "inner rufous" areas upon fore and hind wing. See p. 218.

Fig. 98. The full line represents color-variations of "inner yellow" spot of fore wings in Danaoid Heliconidae. The dotted line represents same for "middle yellow." It is apparent that the "inner yellow" is more variable than the "outer yellow," and also that the variations of both are quite similar to those of the "inner rufous." See p. 219.

Fig. 99. Color-variations of "inner rufous" areas of Acraeoid Heliconidae. The full line represents the fore wing and the dotted line the hind wing. See p. 218.


150
125
100
75
50
25
0
RUFOUS
TRANSLUCENT RUFOUS
TRANSLUCENT LIGHTLY RUFOUS
TRANSPARENT
TRANSLUCENT LIGHTLY YELLOW
TRANSLUCENT YELLOW
YELLOW
BLACK
WHITE

97.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125
100
75
50
25
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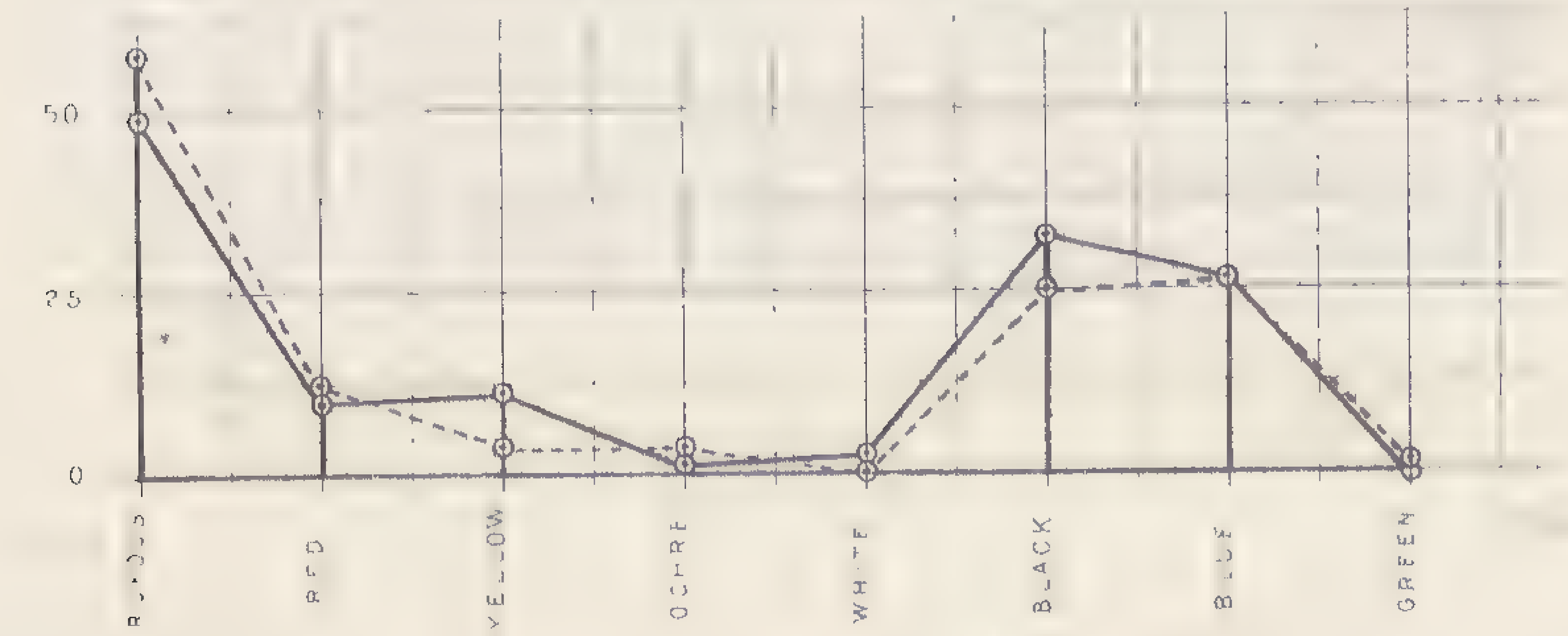
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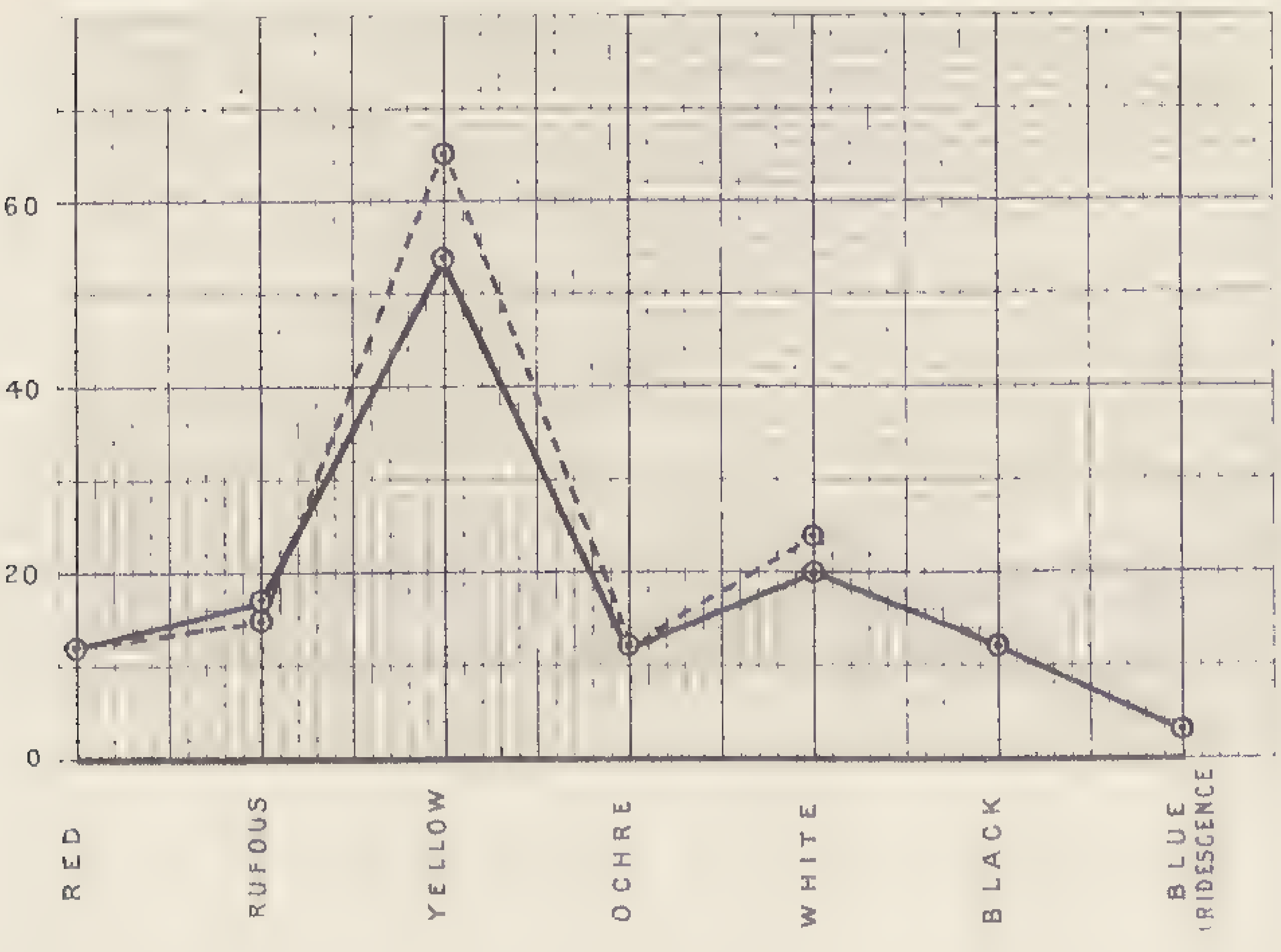
PLATE 10.

Fig. 100. Color-variations of "inner yellow" spots on fore wings of Acraeoid Heliconidae. The full line represents the "inner yellow," the dotted line the "middle yellow." See p. 221.

Fig. 101. Variations of marginal spots upon fore wing in Danaoid Heliconidae. These spots tend to appear either as 2 or 3, or as 6 or 7 spots. See p. 223.

Fig. 102. Variations of marginal spots of hind wings in Danaoid Heliconidae. These spots tend to appear either as 4 or as 5 spots. See p. 223.



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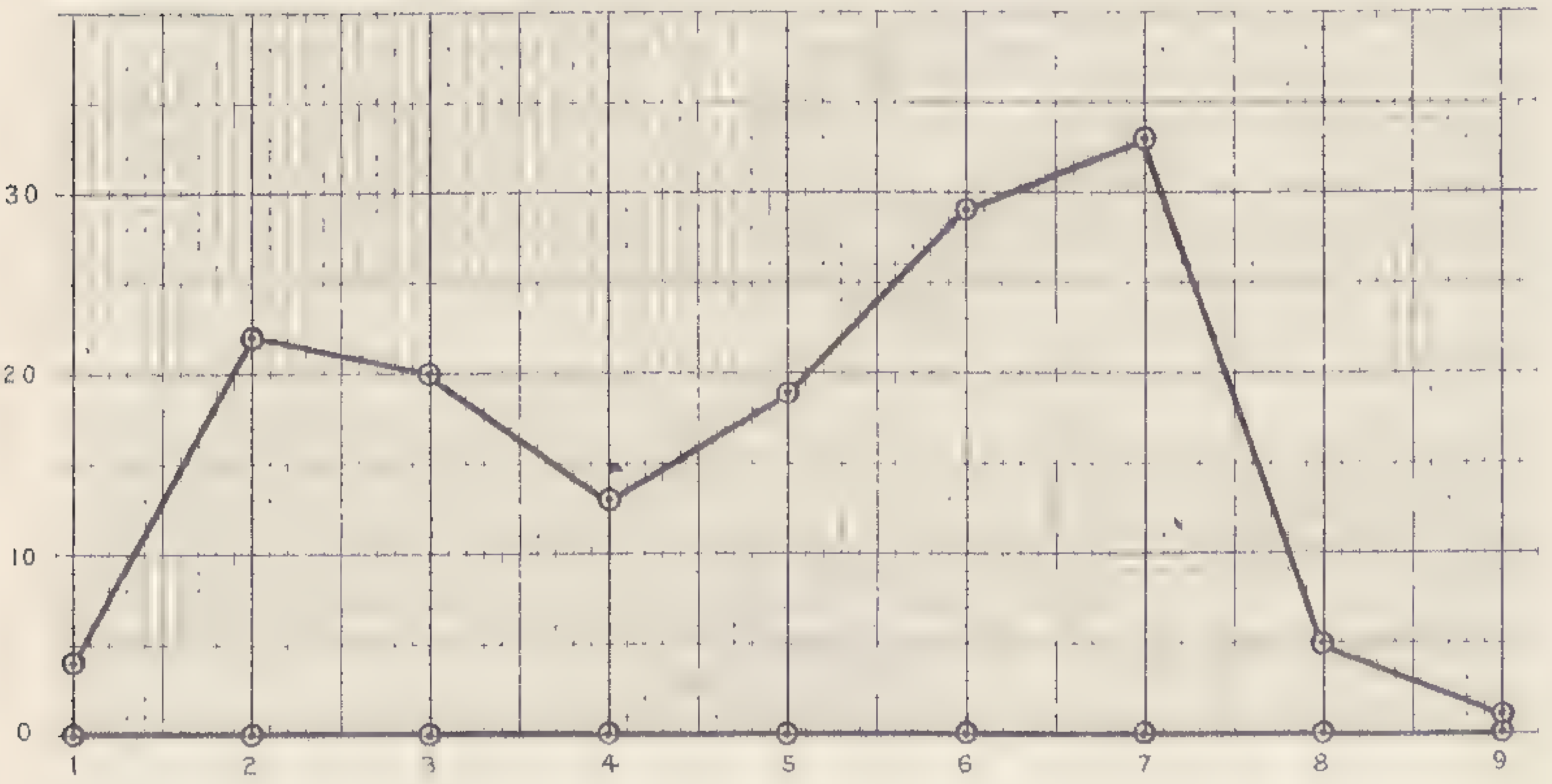

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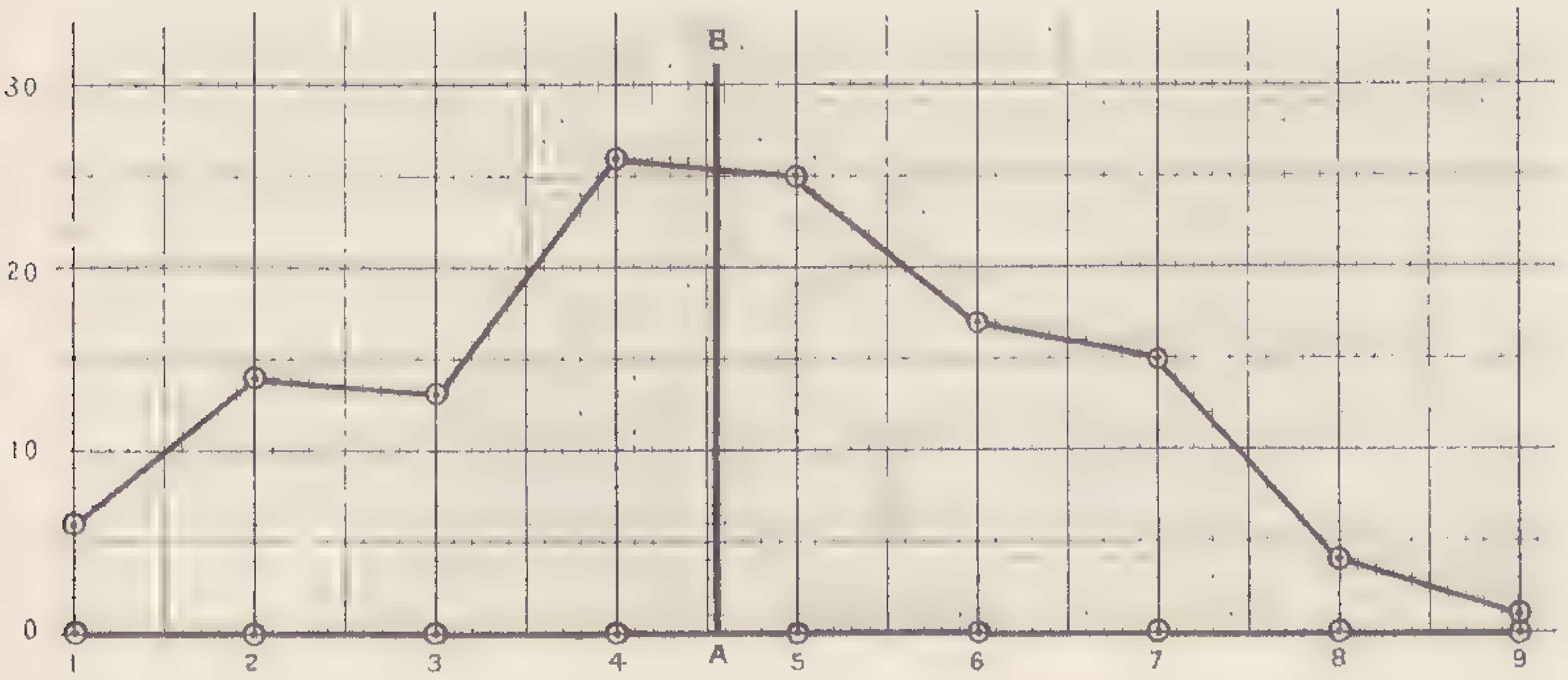

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THE MESENTERIES AND SIPHONOGLYPHS IN METRIDIUM MARGINATUM MILNE-EDWARDS.

BY G. H. PARKER.

WITH ONE PLATE.

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No. 5. — *The Mesenteries and Siphonoglyphs in Metridium marginatum Milne-Edwards.* By G. H. PARKER.[1]

Introduction. — Since the publication of the Hertwigs' ('79) paper on the anatomy of the actinians, the attention of investigators has been more and more directed toward the details of the internal structure of these organisms. This new departure has been conducted in the main on the lines of systematic zoölogy, and, though its advocates in the beginning may have been somewhat Utopian in their expectations, it has certainly carried our understanding of the natural relations of this group of animals a long step forward. The new features thus introduced into the classification have, however, been subject to frequent modification, and every actinian newly investigated may be expected to exert some influence on the classification finally adopted. It is to be regretted that much of this kind of investigation has been of necessity carried out on a limited, often a very limited, number of specimens, so that the possible error of regarding individual variations as characteristic of large groups is not always eliminated.

The following pages contain a record of certain structural peculiarities in a single species of actinian, the common *Metridium marginatum* Milne-Edwards of our coast, as represented by a considerable number of specimens. As this record shows, uniformity of structure is by no means a general characteristic of this species; hence these observations are to some extent a contribution to the study of the variability of this animal.

The material on which the following observations were made consisted of 131 adult specimens of *Metridium marginatum.* These were collected in part by myself and in part by my laboratory assistant, Mr. J. I. Hamaker, to whom I am under obligations for this kindness. All the specimens came from the neighborhood of Newport, R. I., and were prepared and to some extent studied in Mr. Alexander Agassiz's Laboratory at that place. I here wish to express my thanks to Mr. Agassiz for the privilege of carrying on this work at the Newport Laboratory.

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[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, E. L. Mark, Director, No. LXXV.

The specimens were prepared by the Tullberg ('91) method, which consists in stupefaction by the gradual introduction of magnesic sulphate into the water containing the actinians, and in subsequent hardening by means of chromic acid. This method, when properly employed, yields beautifully expanded and thoroughly hardened specimens, and my experience with it has been such that I can fully indorse the recommendations given it by Tullberg ('91), Carlgren ('93, p. 7), and others. Specimens prepared in this way were cut transversely with a common razor, and the number and arrangement of the mesenteries and siphonoglyphs[1] were recorded. Owing to the large size of the specimens, this could be easily done under the magnification of an ordinary hand lens.

Siphonoglyphs. — The Hexactinia, to which Metridium belongs, were until recently supposed to possess always two siphonoglyphs; but this surmise has been shown to be not well grounded, and, in the species under consideration, as McMurrich ('91, p. 131) has already pointed out, either one or two siphonoglyphs may be present. In the 131 specimens that I examined, 77 (or about 59 per cent) had only *one* siphonoglyph (Fig. 3), 53 (or about 41 per cent) had *two* siphonoglyphs (Fig. 1), and a single specimen possessed *three* such organs (Fig. 6). In no instance was a specimen found without a siphonoglyph. The smooth surface of the siphonoglyph is so strongly contrasted with the longitudinally ribbed surface of the rest of the œsophagus that in none of the specimens examined was there any uncertainty as to the number of siphonoglyphs present. The striking difference between these two kinds of surface cannot be made to appear so clearly in the figures as it did in the actual specimen, where, in addition to the cut face, the natural face of the œsophagus could also be inspected. McMurrich ('91, p. 131) remarks that in the individuals examined by him, those with one siphonoglyph were almost, if not quite, as frequent in occurrence as tho with two, but in my enumeration it will be seen that they were really somewhat more numerous.

Since only one of the 131 specimens possessed three siphonoglyphs, it is obvious that this condition may be set aside as distinctly exceptional, and, further, since the other specimens were almost equally divided between those with one and those with two siphonoglyphs, these conditions may fairly be considered typical. It will be convenient in the subsequent discussion to designate these two types by special names, and I shall call that characterized by one siphonoglyph the *monoglyphic* type, and that by two the *diglyphic* type.

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[1] The term "siphonoglyphe" was first introduced into zoölogical nomenclature by Hickson ('84, p. 694), and has since been widely accepted. Professor Hickson kindly informs me that the last syllable of this term is derived from the Greek word γλυφίς, which in the plural form, γλυφίδες, has been used to signify the grooves on an arrow for the insertion of the feathers. The root of this word appears to call for no final *e*, and since in making English words it is best, as Professor Hickson remarks, to use only roots, I therefore propose to change the spelling of the term in question by omitting the final *e*, and to this Professor Hickson assents.

Variations in the number of siphonoglyphs have already been recorded in other actinians. Thus, besides the observations of McMurrich already alluded to, Thorell ('59, Tab. I. Figs. 1 and 2) figured and described specimens of *Metridium dianthus* either with one or with two siphonoglyphs. The monoglyphic condition was also recognized for this species by Gosse ('60, p. 12), who, in ignorance of Thorell's observations, supposed this condition to be characteristic of the species, a mistake afterwards corrected by Foot ('63, p. 64). The presence in some specimens of one, and in others of two siphonoglyphs in *M. dianthus*, as first asserted by Thorell, has recently been confirmed by G. Y. and A. F. Dixon ('91, p. 19), and by Carlgren ('93, p. 104). Furthermore, the Dixons and Carlgren agree in stating that, though two siphonoglyphs may be present in this species, one is the rule. G. Y. and A. F. Dixon ('91, p. 20), moreover, have recorded one specimen of *M. dianthus* with three siphonoglyphs.

Representatives of the genus Sagartia also show variations in the number of their siphonoglyphs; thus G. Y. Dixon ('88, p. 120) observed that in *Sagartia venusta*, *S. nivea*, and *S. mineata*, either one or two siphonoglyphs may be present. The same is probably true of *S. rosea* (cf. F. Dixon, '88, p. 139), and of *S. lactea* (cf. McMurrich, '94, p. 177). In specimens of *Bunodes thallia*, studied by G. Y. and A. F. Dixon ('89, p. 318), one, two, three, and even four siphonoglyphs were observed, although in each of twenty-three adult specimens of *B. verrucosa* the same authors ('89, p. 322) found regularly two siphonoglyphs. Finally Blochmann and Hilger ('88, p. 391) described a specimen of Gonactinia in which traces of a third siphonoglyph seem to have been present.

It is evident from the foregoing account that in several actinians besides Metridium a variation in the number of siphonoglyphs is not unusual, though this variation may not be so pronounced as to constitute a structural type. The importance of these peculiarities from a systematic standpoint has already been appreciated, and in the more recent definitions of the Hexactinia the statement is made that these actinians possess two siphonoglyphs (occasionally one), thereby recognizing the monoglyphic and diglyphic types as normal.

Mesenteries. — In *Metridium marginatum* the pairs of mesenteries are attached lengthwise to the wall of the column, and either reach the œsophagus and unite with it (complete mesenteries) or fall short of that structure (incomplete mesenteries). Of the pairs of complete mesenteries the two usual kinds can be distinguished: those whose longitudinal muscles face the exocœls (directive mesenteries) and those whose longitudinal muscles face the endocœls (non-directive mesenteries).

The *directive mesenteries* are remarkable for the constancy of their relations to the siphonoglyphs. To each siphonoglyph is attached a single pair of directives, and in no instance among the 131 specimens examined was an exception to this rule found. In the monoglyphic type (Figs. 3, 4, 5, 7, and 8) one pair, and only one pair, of directives was present; in the diglyphic type (Figs. 1 and 2) two pairs were invariably observed; and even the single specimen with three siphonoglyphs (Fig. 6) formed no exception, but exhibited three pairs of directives.

This exact correlation between the number of siphonoglyphs and of directives, which probably also obtains in other species of Metridium (cf. Carlgren, '93, p. 106), as well as in the allied genus Sagartia (cf. F. Dixon, '88, p. 136), is rather striking, because the two sets of structures concerned are not invariably thus associated in all actinians. For instance, in Peachia and Oractis (cf. McMurrich, '91, pp. 135, 137), though two pairs of directives are present, only one siphonoglyph occurs; and in Ptychodactis (cf. Appellöf, '94, pp. 5, 7), though two pairs of directives can be seen, no siphonoglyphs are observable. These instances serve to show that in some actinians directive mesenteries may occur without siphonoglyphs, and thus they render more striking the correlation between the variations of the directives and of the siphonoglyphs in *Metridium marginatum.*

The *non-directive mesenteries* vary so much in their number and arrangement that they can best be considered in connection with the particular types with which they occur. In the diglyphic type (53 specimens), in addition to the two pairs of directives, there may be from four to ten pairs of non-directives. The frequency of the occurrence of the different numbers of pairs is indicated in the following table: —

DIGLYPHIC TYPE (two siphonoglyphs and two pairs of directives).

Pairs of Non-directives	4	5	6	7	8	9	10
Number of Cases observed . .	40	4	3	3	2	0	1

In this type (Fig. 1) the two pairs of directives of course divide the non-directives into two groups. I regret that, before it occurred to me to determine the number of mesenteries in each of these two groups for the 53 specimens of this type, some of the specimens were so far dissected as to render them no longer serviceable for this enumeration. I can therefore make a statement concerning this division in the cases of only twenty specimens.

Groups of Non-directives . .	1 + 7	2 + 2	2 + 3	2 + 4	2 + 5	2 + 6	4 + 6
Number of Cases observed .	1	10	2	2	3	1	1

In comparing these results, it will be observed, first, that the great majority of individuals (40 in 53) possess four pairs of non-directives, and, next, that the arrangement of these non-directives in ten cases out of twenty is in two groups of two pairs each. This symmetrical arrangement of the four pairs of non-directives in the diglyphic type reproduces the assumed typical Hexactinian arrangement, and, since the representatives of the other variations are comparatively so few in numbers, this may be taken to be the only characteristic condition of the diglyphic type.

In the monoglyphic type (77 specimens), in addition to the one pair of directives, there were from three to fourteen pairs of non-directives. The frequency of their occurrence is shown in the following table: —

MONOGLYPHIC TYPE (one siphonoglyph and one pair of directives).

Pairs of Non-directives . .	3	4	5	6	7	8	9	10	11	12	13	14
Number of Cases observed .	1	4	20	19	21	5	1	4	1	0	0	1

Admitting the monoglyphic type to be derived from the diglyphic by the conversion of a pair of directives into a pair of non-directives, one would expect the monoglyphic type with five pairs of directives to be most often met with. Such, however, is not the case, for specimens with six or seven pairs of non-directives are about as numerous as those with five. Since any of the three groups with five, six, or seven pairs of non-directives is represented by a greater number of individuals than all the other minor groups of variations taken collectively (cf. Table of Monoglyphic Type), it is clear that in the monoglyphic type there are three structural subtypes characterized respectively by five, six, and seven pairs of non-directives, instead of only a single such subtype, as in the diglyphic condition. These relations indicate a certain degree of distinctness between the diglyphic and the monoglyphic type; for the monoglyphic has obviously a greater range in variation, as shown in its three subtypes, than the diglyphic with only a single one. It is an interesting fact in this connection, that the monoglyphic subtype with six pairs of non-directives often repeats (Fig. 4), so far as its complete mesenteries are concerned, the arrangement of mesenteries found in Scytophorus, for which R. Hertwig ('82, p. 104) constructed a separate family, the Monauleæ.

It might at first be suspected that the three monoglyphic subtypes pointed out above, and in fact all the variations in the number of complete mesenteries, could be explained on the assumption that certain incomplete mesenteries by excessive growth had become complete, or that complete ones had become incomplete, thus introducing a variation in the number of complete mesenteries, without, however, altering the total number of all kinds of mesenteries; but in the individuals examined the relative development of the incomplete mesenteries was found to be subject to so much variation that the satisfactory determination of the total number of mesenteries as a basis of comparison was practically impossible, and all attempts to carry through interpretations such as that suggested above resulted in such ambiguous and strained results that the unnaturalness of the method condemned it. Moreover, in the monoglyphic type with *six* pairs of non-directives (Fig. 4), incomplete as well as complete mesenteries are sometimes so symmetrically placed that no attempt to readjust them is warranted. What may be said of such cases is, that, in place of the usual five pairs of non-directives, six pairs are present, and this increase cannot be ascribed to reinforcement from the ranks of incomplete mesenteries. Such cases as these are so frequent, and instances that may be interpreted as the conversion of complete into incomplete mesenteries or the reverse are so few, that it must be admitted, I believe, that these differences are due much more frequently to fundamental differences in the plans on which the mesenteries of different individuals are laid down than to the more easily conceived relation between complete and incomplete mesenteries.

The *incomplete mesenteries* have not been exhaustively investigated. Their great number, variability in size, and the frequent difficulty met with in attempting to classify them, render such a task nearly impossible. In what are generally assumed to be the more typical specimens of Metridium (Fig. 1), an exocœl may contain one pair of secondary mesenteries, two pairs of tertiaries, four pairs of quarternaries, and evidences (ridges) of eight pairs of quinaries. Though this condition was occasionally realized, in the great majority of cases irregularities in what are presumably secondaries and tertiaries, not to mention higher orders, were so numerous that consistent tabulation was out of the question. So far as size and position were concerned, what seemed to be secondaries showed such variations that no two specimens in which the arrangement of the complete mesenteries agreed, had similar arrangements of the secondaries, except in six instances of the 40 typical diglyphic specimens; and each of these six instances showed variations in the tertiaries characteristic of it as an individual. So far, then, as the incomplete mesenteries are concerned, we soon reach groups of variations by which individuals may be characterized; in other words, if the variations of the primaries (complete mesenteries), secondaries, and tertiaries be considered together, it will be seen that no two of the 131 specimens examined were alike, each one having a combination of variations peculiar to itself. This is, perhaps, the most important feature in the variations of the incomplete mesenteries.

That variations in the number of mesenteries, such as have been pointed out in the preceding paragraphs, occur in other actinians is well known. Thus Carlgren ('93, p. 106) states that in *Metridium dianthus*, in addition to a single pair of directives, six, seven, or even nine pairs of non-directives may occur, and F. Dixon ('88) has shown that in several species of Sagartia the number of non-directives may reach twelve or even sixteen pairs. Further, in four specimens of *Bunodes thallia*, G. Y. and A. F. Dixon ('89, pp. 317, 318) found respectively 15, 19, 21, and 26 pairs of non-directives. These citations suffice to show that extensive variations in the mesenteries may occur in other actinians than *Metridium marginatum*, but the cases recorded for any one species are so few that generalizations cannot be drawn from them.

As a rule, variations in the mesenteries occur in both members of a pair in the same way, but not infrequently one finds pairs in which the two members are not equally developed. When this occurs amongst the complete or nearly complete mesenteries, it may result in the formation of a pair one member of which is complete and the other incomplete (Fig. 2). The 131 specimens of Metridium examined possessed in all 739 pairs of non-directives, and, of these, 17 pairs (or about 2.4 per cent), distributed through thirteen individuals, possessed each an incomplete member. Of the thirteen individuals exhibiting this variation, ten were of the monoglyphic type, and three of the diglyphic type. In the monoglyphic type it is customary to assume that the single siphonoglyph present corresponds to the so-called ventral one of the diglyphic condition. This assumption is at least convenient, for it allows us to distinguish in each pair of lateral non-directives a dorsal and a ventral member. Admitting this distinction for the sake of description, it may be said that seven of the ten monoglyphic specimens had each a single pair of non-directives in which one member was incomplete, and of these incomplete mesenteries four were dorsal, two were ventral, and one was indeterminable (Fig. 8); and that the three remaining monoglyphic specimens had each two such pairs, of which in one instance both the incomplete mesenteries were dorsal, and in two instances one was a dorsal and the other a ventral mesentery (Fig. 7). Thus in the ten monoglyphic specimens, this variation was observed in thirteen pairs of mesenteries, of which eight presented incomplete dorsal members, four incomplete ventral members, and one was indeterminable. It is evident that this variation is not limited to either dorsal or ventral members, and is not correlated with the fact that in many actinians ventral members, as a rule, develop later than dorsal ones; in other words, this variation is probably not to be regarded as atavistic.

In the adult condition of the diglyphic type, I see no way of distinguishing dorsal from ventral, and the most that can be said of the three cases of variation met with under this type is that in two of them only one mesentery each was incomplete (Fig. 2), while in the third two were incomplete. In the latter case the two mesenteries (as in Fig. 7) were not on corresponding sides; hence one of them must have been dorsal and the other ventral, but exact determination could not be made. The variations in the diglyphic type, then, present no essential features not already met with in the monoglyphic type.

Many pairs of mesenteries in which both members are incomplete show variation of the kind indicated above, in that one member is larger than the other (Fig. 4), but because of the extreme variability of these parts no record has been kept of such variations.

In a few cases *single* mesenteries have been observed (Fig. 2). These, as the arrangement of the longitudinal muscles of their neighbors shows, have absolutely no trace of a mate. In the instance figured, it is difficult to decide which of the two mesenteries, the complete (y) or the incomplete (x), is the single one. One or other must be. Single mesenteries as exceptions have already been recorded by F. Dixon ('88, p. 138) in Sagartia, and by Carlgren ('93, p. 106) in Metridium.

Among the complete mesenteries, two cases of union by what would have been the median margins of the participants have been observed (Fig. 8). An instance of this kind has already been recorded by R. Hertwig ('82, p. 37) in Tealia, and in this, as in Metridium, the united mesenteries were not members of the same pair, but of adjacent pairs.

No instances of the occurrence of longitudinal muscles on both the exocœl and the endocœl face of the same mesentery, as observed by McMurrich ('89, p. 30) in Aulactinia, have been noticed.

So far as the mutual arrangement of complete and incomplete mesenteries is concerned, the monoglyphic and diglyphic types show rather characteristic differences. In the diglyphic type the complete mesenteries usually show no special tendency to collect at one pole or the other of the animal (cf. Fig. 1). In the monoglyphic type there is often a marked tendency for all but two pairs of the non-directives to collect opposite the directives (cf. Fig. 5); consequently the half of the animal centering about the directives has an arrangement of parts like that found in the corresponding half of a diglyphic animal, while the other half contains a more or less crowded group of non-directives. In this respect Metridium seems to differ from Sagartia, in which, according to the figures given by F. Dixon ('88, Plate I.), such a crowding of non-directives is not noticeable. This condition recalls in a superficial way that found in Cerianthus, in which an active growth of mesenteries takes place opposite the siphonoglyph.

The characteristic arrangements of the mesenteries in connection with the monoglyphic and diglyphic types probably recur under similar conditions in *M. dianthus;* for such arrangements have been figured by Thorell ('59, Tab. I. Figs. 1 and 2) and briefly described by Carlgren ('93, p. 106).

While the crowding of the mesenteries occurs as a rule only in monoglyphic specimens of Metridium, one instance of it has been observed in a diglyphic specimen (Fig. 2), and here the general resemblance to the specimen with three siphonoglyphs (Fig. 6) is so striking that I have felt almost justified in interpreting this specimen as a triglyphic animal, at one pole of which the directives, with the loss of the siphonoglyph, had given place to a group of non-directives.

In the preceding account I have intentionally avoided, as far as possible, the use of the terms dorsal and ventral as applied to the two poles of the actinian's body. This has not been because of objections that might well have been raised against these terms in themselves, as Haddon ('89, p. 300) has done, but because of the more fundamental question of whether dorsal and ventral can really be distinguished in an adult Metridium. These terms, as is well known, may be applied with perfect precision to the adults of forms like Edwardsia, where the longitudinal muscles bear very unlike relations to the two poles of the animal; but in forms like the diglyphic type of Metridium (Fig. 1), where the muscles of the pairs of non-directives are similarly related to both poles, this means of distinguishing dorsal and ventral is lost. It has been suggested that even in cases of this kind dorsal and ventral may still be distinguished, either by the conditions of the siphonoglyphs, — the ventral being better developed than the dorsal (Faurot, '95, p. 62), — or by the condition of the subsidiary mesenteries, — the more dorsal pairs, because of their earlier development, remaining larger than the ventral ones (Carlgren, '93, p. 100). Unfortunately, these criteria, even supposing them to be true, which is by no means certain, cannot be employed on the diglyphic type of Metridium because of the similarity of its two poles. So far as the adult diglyphic Metridium is concerned, I am obliged to confess that I can find no satisfactory criteria for the determination of dorsal and ventral relations.

With the monoglyphic type the case seems simpler. It is generally stated that, when only one siphonoglyph is present, it is the ventral one; but, as Carlgren ('93, p. 100) remarks, so far as Sagartia is concerned, this statement has never been accompanied with any direct proof; nor, I may also add, has it been proved for Metridium. The argument used by McMurrich ('91, p. 133) to show that the single siphonoglyph in the monoglyphic Metridium is the ventral one may be used with equal accuracy to show that this siphonoglyph is the dorsal one, for the argument advanced rests upon the sequence of the development of the mesenteries, which, being unknown in Metridium, has simply been assumed by McMurrich. The case of Metridium seems to be precisely like that of

Sagartia, in which, as Haddon ('89, p. 300) remarks, it seems impossible, in our present state of knowledge, to determine dorsal and ventral relations. It is probable that this determination can be made only after the sequence of development of these mesenteries has been discovered. In the four types of sequence thus far known (cf. Fowler, '94, p. 470), the ventral directives are always the third pair of mesenteries to form, and the dorsal directives either the second or fourth. It is probable that, when the developmental sequence of the mesenteries is discovered for the two types of Metridium, the determination of dorsal and ventral in this actinian will be made with as much certainty as in any other, and we shall probably then know whether in the monoglyphic type the single siphonoglyph is a dorsal one, a ventral one, or in some specimens one and in others the other.

Before concluding this account of the mesenteries in Metridium, I wish to consider briefly some other aspects of the monoglyphic and diglyphic types. When I first perceived that there were two structural types in Metridium, I suspected that they might be correlated with sexual differences. To test this question, I determined the sexes of a number of individuals of each type. In ten monoglyphic specimens, five were females and five were males; in twenty-seven diglyphic specimens, fifteen were females and twelve were males. Evidently the two types are not correlated with difference in sex.

The fact that the two sexes occur in about equal numbers under both types suggests that these two types may in reality be two varieties of the species *Metridium marginatum*. In support of this opinion, it may be mentioned that the two types show a difference in the degree of their variability, the diglyphic type having only one subtype, the monoglyphic three; and, further, that, while the diglyphic type presents usually a rather typical Hexactinian arrangement of mesenteries, the monoglyphic type shows a general tendency to crowd the non-directive mesenteries to the region opposite the one siphonoglyph.

These differences, however, fairly marked as they are, are insufficient in my opinion to warrant the assumption that the two types are really varieties, and the determination of this question must wait, I believe, till more is known of the breeding habits of Metridium. If it can be shown that in the offspring of one animal representatives of both types occur, the idea that we are dealing with varieties could not be maintained, and the species could at most be said to be dimorphic. If, however, the types could be shown to breed true, they might with justice be described as varieties.

Should these types prove not to be of the value of varieties, they may still possibly be correlated with the methods of reproduction. Besides the sexual method, *Metridium marginatum* reproduces non-sexually by small buds cut off from the margin of the animal between its aboral disk and its column. This method of reproduction, long ago hinted at by Verrill ('69, p. 257), can usually be seen taking place in any large specimen. Similar conditions have been observed in *Metridium dianthus* by G. Y. and A. F. Dixon ('91, p. 20), and by Carlgren ('93, p. 108). Possibly the two types here described are the products, one of the sexual, the other of the non-sexual, method of reproduction. The solution of this question, however, must be left to future investigation.

Cambridge, January 9, 1897.

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EXPLANATION OF PLATE.

All figures represent transverse sections of the column of *Metridium marginatum* Milne-Edwards. In each case all the complete mesenteries have been drawn, and in some instances a few of the incomplete ones. All figures are magnified about 1.5 diameters.

Fig. 1. Diglyphic specimen, showing typical hexamerous arrangement of the complete mesenteries, and a regular group of subordinate mesenteries in a primary exocœl.

Fig. 2. Diglyphic specimen, showing a very irregular arrangement of the complete mesenteries, one group of which resembles the crowded group of mesenteries found opposite the directive pole in the monoglyphic type. In this crowded group occurs an unpaired mesentery (x or y).

Fig. 3. Monoglyphic specimen, with five pairs of non-directives, showing the regular arrangement of the subordinate mesenteries about the pair of non-directives opposite the directive pole.

Fig. 4. Monoglyphic specimen, with six pairs of non-directives, showing the regular arrangement of the subordinate mesenteries about the two pairs of non-directives opposite the directive pole.

Fig. 5. Monoglyphic specimen, with ten pairs of non-directives, showing the crowding of the non-directives in the region opposite the directive pole.

Fig. 6. Triglyphic specimen.

Fig. 7. Monoglyphic specimen, showing pairs of mesenteries in which dorsal as well as ventral components are incomplete.

Fig. 8. Monoglyphic specimen, showing the union of two primary mesenteries.

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PHOTOMECHANICAL CHANGES IN THE RETINAL PIGMENT CELLS OF PALÆMONETES, AND THEIR RELATION TO THE CENTRAL NERVOUS SYSTEM.

BY G. H. PARKER.

WITH ONE PLATE.

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No. 6. — *Photomechanical Changes in the Retinal Pigment Cells of Palæmonetes, and their Relation to the Central Nervous System.*

By G. H. Parker.[1]

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Introduction.

The present paper is a record of a series of experiments on the photomechanical changes in the pigment cells of the retina in *Palæmonetes vulgaris* Stimp. This species is especially favorable for such work, since its retina exhibits in a marked degree all the kinds of pigment changes that have thus far been observed in the eyes of crustaceans.

Those that have worked upon this subject have, in the main, followed in the lines laid down by Boll, Engelmann, and others in their studies on the eyes of vertebrates. Although the pigment changes in vertebrates are relatively simple, they are, even now, far from being satisfactorily understood, and it is therefore not surprising that in the arthropods, where the pigment changes of the retina are probably more complex than in any other group of animals, much still remains to be done. There has been a tendency, moreover, among some of those that have studied such phenomena, to generalize on observations taken from eyes of totally different types, such as the compound eyes of insects and the simple eyes of arachnids; and this tendency, though in a measure justifiable, has led, I believe, to a want of attention to the characteristic differences of the pigment changes in each given type of eye, a matter that, in my opinion, lies at the foundation of any satisfactory understanding of these changes. What is most needed at present, therefore, seems to be a thorough and exhaustive study of the pigment changes of each of the more important types, rather than an inspection, necessarily more or less superficial, of the various arthropod eyes that have not as yet been examined. The following studies have been made with the hope that they would contribute in this respect to a more complete understanding of the pigment changes in the compound eye, especially in crustaceans.

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[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, E. L. Mark, Director, No. LXXVI.

The earliest paper on the pigment changes in compound eyes, so far as I am aware, was published in 1889 by Exner ('89), and contains in a condensed form the essential peculiarities of the pigment changes in the compound eyes of certain insects. In 1890 Stefanowska ('90) published an account in which this subject was again considered, but with a wider range of material. In the next year three contributions appeared: Exner's ('91) brilliant and important essay on the physiology of compound eyes, of which his former publication had been in the nature of a partial preliminary notice; Szczawinska's ('91) article on the pigment changes in the eyes of crustaceans and arachnids; and Herrick's ('91) account of similar changes in the eyes of Palæmonetes, contained in his monograph on the development of Alpheus. Three years later Kiesel ('94) described some very noteworthy observations on the pigment changes in the eyes of insects. The following year the writer (Parker, '95) published, in connection with other matters, an account of the retinal pigment changes in Astacus, and a preliminary statement of the results given in full in this paper was published last year (Parker, '96). These, I believe, are all the publications in which the questions here raised have been considered. Critical comments on their contents will be found in the following pages.

Structure of the Eye in Palæmonetes.

Before describing the pigment changes in the retina of Palæmonetes, it will be necessary to outline briefly the structure of the eye in this animal. The eye may be said to be that portion of the optic apparatus contained in the optic stalk. It consists of a retina, at the distal end of the stalk, and a series of four optic ganglia, which extend through the axial portion of the stalk. The retina is connected with the first optic ganglion by the retinal nerve fibres. Nerve fibres connect the first optic ganglion with the second, the second with the third, and the third with the fourth. From the fourth optic ganglion, which is situated near the proximal end of the stalk, the optic nerve extends to the brain. The finer structure of the optic ganglia in Palæmonetes is in all probability essentially the same as in Astacus, where, as I have already shown (Parker, '95, Taf. 3, Fig. 59), each optic ganglion represents a region of interruption for the great majority of the nerve fibres that intervene between the retina and the brain.

The retina in Palæmonetes is composed of ommatidia, the structure of which has already been described at length (Parker, '91, p. 108, Pl. IX.). For convenience I add a brief summary of this description. Each ommatidium is composed of five kinds of cells. Immediately under the corneal facet (Fig. 1, *crn.*) are two corneal hypodermal cells (*nl. crn.*). The distal portion of the axis of the ommatidium is occupied by the cone (*con.*), which, as seen in transverse sections (Fig. 3, *cl. con.*), is composed of four parts. Each part contains near its distal end a nucleus (Fig. 1, *nl. con.*) and represents a cell. The four cone cells are closely applied to one another in the region of the cone proper (Fig. 3). Proximally they taper off as thick, more or less independent fibres. (Compare Figs. 1, 4, and 5.) These fibres separate and apparently terminate near the distal end of the rhabdome (Fig. 1, *rhb.*). I have been unable to trace them further, though I suspected that they might end, as in Homarus (Parker, '90, p. 14), on the basement membrane. The distal retinular cells either apply themselves to the lower portion of the sides of the cone (Fig. 2, *cl. dst.*), in which case they are so closely packed that their outlines cannot be distinguished (Fig. 3, *cl. dst.*), or they occupy a more proximal position (Fig. 1, *cl. dst.*), forming a ring around the attenuated ends of the cone cells (Fig. 5). There is, of course, one ring for each ommatidium. Each ring contains six distal retinular cells, but these rings are so constituted that each cell is at the same time a member of three rings; hence there are in reality only twice as many distal retinular cells as there are ommatidia. The proximal portion of the axis of the ommatidium is occupied by the rhabdome (Fig. 1, *rhb.*), which is surrounded by seven functional proximal retinular cells (Fig. 6, *cl. px.*), in addition to which an eighth rudimentary one is present (Parker, '91, p. 111). Each functional cell ends distally in a somewhat swollen knob containing its nucleus (Fig. 1, *nl. px.*). From this swollen end the cell extends proximally over the rhabdome, beyond which it becomes slightly attenuated, and, as a retinal nerve fibre (Figs. 1 and 7, *fbr. r.*), pierces the basement membrane (*mb. ba.*) and extends to the first optic ganglion. Here it probably terminates in a fibrillation, as has already been shown to be the case in Astacus (Parker, '95, p. 41). The accessory pigment cells (Fig. 1, *cl. sn.*) occupy the space in the deeper part of the retina. The number of these cells is not constant, but, judging from their nuclei, it is not more than one or two for each ommatidium. Proximal processes extend from these cells through the apertures in the basement membrane to the distal surface of the first optic ganglion, and distal processes may extend forward to the front faces of the distal retinular cells. Each ommatidium in Palæmonetes, then, is composed of the following cells: two corneal hypodermal cells, four cone cells, two distal retinular cells, eight proximal retinular cells (one of which is rudimentary), and a variable but small number of accessory pigment cells. Black pigment granules are contained in both the distal and the proximal retinular cells, and are limited to these cells; the whitish pigment lies exclusively in the accessory pigment cells. The seven functional proximal retinular cells are the only elements of the ommatidium that are known to have nervous connections. These brief anatomical statements may suffice as an introduction to the consideration of the pigment changes in the retina.

Photomechanical Changes in Normal Retina.

The general *method* by which the normal photomechanical action of the retinal pigment cells in Palæmonetes was determined consisted in the examination of eyes that had been kept in the light or in the dark known periods of time. For a dark chamber I used a box with a tight-fitting cover. From time to time during the course of the experiments this box was tested for its light-proof qualities by exposing in it a very sensitive bromide paper, such as is used by photographers. In all my experiments this showed complete absence of light. The top of the box was pierced by a hole, through which a piece of rubber tubing was introduced so that fluids could be poured into the box without exposing its contents to light. Two or three turns in this tube were found sufficient to prevent such light as entered the outer end of the tube from reaching the interior of the box. Living shrimps in a vessel of water were placed in the box, and the cover was carefully closed. After the expiration of the required interval, hot water was run in through the tube, and the animals were thus killed in the dark. Other killing reagents, such as corrosive sublimate, picric acid, etc., were tried, but none proved so satisfactory as water at about 80° C. The periods of exposure to dark in the first set of experiments were as follows: 1 min., 5 min., 10 min., 15 min., 30 min., 45 min., 60 min., and then at intervals of an hour up to 8 hours. It was found subsequently that the experiments need not have extended over a maximum period of more than two hours, and that intervals of about fifteen minutes were all that were needed to observe the steps of the change. From each lot of animals prepared in this way, the optic stalks were cut into sections for examination under the microscope. In a similar way, the eyes of animals that had been kept some four hours in the dark were exposed to the light for given intervals, killed, cut, and examined. In cases where it was necessary to make very accurate comparisons, the eyes of the same animal were used for the two conditions; thus, after keeping the animal a given time in the light, one optic stalk was removed, and the animal kept in the dark. At the expiration of the second interval, the second optic stalk was removed and prepared. To guard against individual variations, in every experiment the eyes of at least three animals were examined.

The only *general changes* shown by retinas subjected to light or dark were changes in the arrangement of the pigment. In other respects they were not noticeably altered. Thus, no change in thickness was observable; in one case, a left retina that had been kept in the dark measured in its middle region from the corneal cuticula to the basement membrane 263 μ, while the right retina from the same animal exposed to light measured 270 μ. In a second case, a dark left retina measured 240 μ, the light right one measuring 233 μ. The cones likewise showed no significant differences. By analogy with the perceptive elements in the vertebrate eye, one might have expected the rhabdomes, the terminal nervous organs of the crustacean eye, to shorten in the light and lengthen in the dark. I was unable to obtain evidence of such a change in Palæmonetes, and yet the conditions for the exact measurement of the rhabdomes are so unfavorable in this animal that I am by no means certain that these changes may not occur. If, however, they do take place, they must be relatively small. The observable changes induced in the retina by the absence or presence of light affect the three kinds of pigment cells, — the proximal retinular cells, the accessory cells, and the distal retinular cells. These will be considered in the order given.

The pigment in the *proximal retinular cells* forms at the base of the retina a band, called by Exner ('91, p. 62) the retinal pigment. The photomechanical changes that this pigment undergoes have already been observed in various crustaceans by Exner ('91), Szczawinska ('91), and myself (Parker, '95); and, so far as the chief facts of these changes are concerned, the accounts given by these writers are in substantial agreement. In no case, however, has the precise character of these changes been followed, nor the time needed for their completion been recorded.

In a proximal retinular cell that shows the full effect of light (Fig. 1), the black pigment granules are almost uniformly scattered from the distal end of the cell backward through its whole length, including the retinal nerve fibre, to the region of the first optic ganglion. In the body of the cell proper (Fig. 6), as well as in the retinal nerve fibre (Fig. 7), it will be observed that the pigment granules lie entirely within the limits of these structures; in other words, the black pigment of this portion of the retina is contained entirely within the proximal retinular cells. This pigment, though in the main uniformly distributed through the cell, shows regularly two slight concentrations, — one at the swollen distal end of the cell (Fig. 1), and another on the sides of the rhabdome. Small irregular concentrations may also occur in the body of the cell. In the eye subjected to light, the only part of the cell except the nucleus that is entirely free from pigment granules is a transparent axis that can be traced from the region of the rhabdome down through the body of the cell, and through the whole length of the retinal nerve fibre. This is undoubtedly the axis cylinder of the nerve fibre, which, in its passage to the rhabdome, extends through the body of the cell.

In crustaceans like Cancer (Parker, '91, p. 116, Plate X. Fig. 131), in which the proximal retinular cells are more fully provided with pigment granules than in Palæmonetes, this axis is more conspicuous.

In an eye that has been kept in the dark for several hours, the bodies of the proximal retinular cells are without trace of pigment (Fig. 2), the whole mass of black pigment being concentrated in the retinal nerve fibres, i. e. proximal to the basement membrane. Here, as in the former case, the pigment lies entirely within the limits of the retinular cell.

The transition from the dark condition to the light condition of the eye was accomplished by the following steps. In an eye that had been kept some four hours in the dark and then exposed for five minutes to the light, the arrangement of the pigment in the proximal retinular cells was indistinguishable from that characteristic for full darkness. After ten minutes' exposure to light, the pigment was found to have moved forwards to the level of the basement membrane. After fifteen minutes, it was found throughout the bodies of the cells; and, at thirty minutes, well marked concentrations had appeared about the rhabdome and at the distal end of each cell. At forty-five minutes, these concentrations were somewhat more pronounced, but after that time no further changes were observable.

The reverse change, which takes place in the dark, is accomplished in the following manner. After the animal has been in the dark fifteen minutes, the concentrations of pigment about the rhabdomes and at the distal ends of the cells have almost disappeared, though the bodies of the cells still contain an almost uniform amount of pigment throughout their whole length. After thirty minutes, much more pigment is to be found proximal to the basement membrane than distal to it, and after forty-five minutes almost all the pigment is proximal in position. At the end of an hour, the condition characteristic of darkness is fully realized.

The changes just recorded occur entirely within the limits of each proximal retinular cell. There is no reason for believing that the changes are the results of a process of pigment production in one part of the cell, and of pigment destruction in another. The observed facts, on the contrary, suggest that the pigment granules of one region in the cell are moved to another. The movement, however, is not accompanied by any noticeable change in the position or even the form of the containing cell. The pigment granules seem to be carried up and down through the cell, as though by a streaming of the cell protoplasm. A similar stability of form, accompanied with an internal movement of pigment, has been described by Ballowitz ('93, Taf. XXXVI. Fig. 12, and '93^a, p. 629) in the pigment cells of the skin of fishes.

Through the kindness of Professor F. H. Herrick, I have had the privilege of examining an interesting series of eyes taken from specimens of Palæmonetes that had been kept living in a dark chamber thirty-eight days. The pigment in the proximal retinular cells of such animals showed the condition characteristic of the dark. In an animal that had been kept in the dark for this period and then exposed to light for four hours and three quarters, the pigment returned partially to the position characteristic of the light. The greater part of it remained proximal to the basement membrane, and from that which moved into the bodies of the cells no marked concentrations were formed, either about the rhabdomes or at the distal ends of the cells. Long confinement in the dark, then, seems to interfere somewhat with the mechanism by which the pigment of these cells is normally moved.

The *accessory pigment cells* are located in the base of the retina, and send a few processes distad to the outer surfaces of the distal retinular cells, and many proximad through the apertures in the basement membrane to the distal surface of the first optic ganglion. The pigment with which these cells seem to be almost entirely filled is yellowish by transmitted light, and white by reflected light. It is especially remarkable for its powers of reflecting light, and this quality led Exner ('91, p. 97) to designate the layer formed from it by the name of the tapetum. Whether this pigment is influenced by the presence or absence of light is a matter of some uncertainty. Szczawinska ('91, p. 552) states that in Astacus, under the influence of light, the cells containing it enlarge slightly. Exner ('91, p. 105), though at first inclined to regard the accessory cells as influenced by the light, was finally led to abandon this view, and to explain their two apparent conditions by the greater or less degree with which they were covered by the migrating pigment of the proximal retinular cells. In a preparation from an eye kept in the dark, the retinular pigment, as already mentioned, is entirely below the basement membrane, and the accessory pigment is almost entirely exposed, and consequently conspicuous. In the light it is somewhat covered by the black pigment, which under these circumstances fills the bodies of the proximal retinular cells, and it thus becomes less noticeable than before. My own studies on the retina of Astacus (Parker '95, p. 25) led me to agree with Exner that the accessory pigment showed only an apparent change. If, however, any change did occur, it was certainly not an increase in the size (conspicuousness?) of the accessory cells under the action of light, as maintained by Szczawinska, but rather the reverse.

Although in respect to Astacus I am still in doubt as to whether or not the accessory cells show any photomechanical changes, I have not the least hesitancy in stating that in Palæmonetes such a change does occur. The principal difficulty in demonstrating this change comes from the disturbing influence produced by the migration of the pigment in the proximal retinular cells. This difficulty, however, can be overcome by the employment of a depigmenting reagent that will remove the retinular pigment without affecting the accessory pigment. Such a reagent is the depigmenting fluid recommended by Grenacher ('86, p. 214). In preparations representing the dark and the light condition, and depigmented by this means, the differences in the distribution of the pigment in the accessory pigment cells is so striking that no one would question for a moment the photomechanical activities of these cells. In the light (Fig. 1) the accessory pigment forms two concentrations, one in the base of the retina, and the other near the distal surface of the first optic ganglion. These two concentrations are connected by irregular bands of pigment. In the dark (Fig. 2) almost all the accessory pigment is in the base of the retina, the concentration near the ganglion as well as the intermediate pigmented bands being represented by only a few small pigmented patches.

The change from the condition produced by the light to that produced in the dark is indicated in the following steps. After the animal has been about thirty minutes in the dark, the concentration of pigment formerly near the optic ganglion is appreciably nearer the retina. After forty-five minutes, this concentration as such has disappeared, and that in the retina has considerably increased. Finally, after two hours, almost all the accessory pigment lies in the base of the retina, there being only a few small strands proximal to the basement membrane.

In the reverse change under the influence of light, the intermediate pigment strands show a perceptible thickening between ten and fifteen minutes after the eye has been placed in the light, and the full concentration at the level of the ganglion is completed within the period extending from forty-five minutes to an hour after that event.

I have never been able to discover any outlines to the accessory pigment cells except those indicated by the pigment mass itself. Judging from these, the photomechanical changes in the accessory cells involve so radical an alteration in the forms of the cells that the latter may be said to have assumed a different position. In this respect, then, the pigment changes in these cells involve much more active movements than in the case of the proximal retinular cells, and possess something of a locomotor character. So far as I have observed them, they may be compared with perfect propriety to the more or less circumscribed movements of an amœba. When the retina is placed in the light, the cells with their contained pigment creep slowly backward through the apertures in the basement membrane toward the optic ganglion. When the retina is in the dark, they reverse this movement and creep out into the base of the retina. The one particular in which this movement differs from that of an amœba is that of its limitations in direction. Thus the cells always creep either outward or inward. Moreover, in darkness they do not creep indefinitely outward, but after about two hours reach a maximum limit; the same is true of their inward course. These limitations may be due either to the structure of the regions into which the cells creep, or to the intrinsic qualities of the cells themselves; but I have been unable to get conclusive evidence as to which it is.

In the interesting series of eyes of Palæmonetes loaned me by Professor Herrick, the accessory pigment cells of the eyes that had been kept in the dark thirty-eight days presented a condition normal for exposure to the dark. In those eyes that had afterwards been exposed to the light for four hours and three quarters, this pigment had apparently resumed the position normal for exposure to light. The mechanism by which the accessory pigment changes are brought about, unlike that for the proximal retinular pigment changes, is therefore apparently not interfered with by prolonged retention in the dark.

The *distal retinular cells* present photomechanical changes more complex than those in the two kinds of cells already considered. These changes have been described by Exner ('89 and '91), Szczawinska ('91), Herrick ('91), and myself (Parker, '95). All investigators are agreed, I believe, in stating that in the dark these cells occupy a more distal position than in the light. Their probable influence on the amount of effective light that enters the retina led Exner ('91, p. 63) to call them the iris pigment. In Palæmonetes, as I have already shown, there are two distal retinular cells for each ommatidium.

In an animal that has been subjected to the full action of light, the distal retinular cells (Fig. 1, *cl. dst.*) are plump ovoid bodies in contact with the outer ends of the proximal retinular cells. The body of each distal cell has the length of about $30\,\mu$. From its outer end a single process usually extends to, or at least toward, the corneal hypodermis. The whole distal retinular cell, excepting its nucleus and sometimes a portion of its distal process, is filled with black pigment. The whitish pigment that often occurs on the outer surface of these cells represents, as already mentioned, a distal process from the accessory pigment cells.

In animals kept a sufficient time in the dark, the bodies of the distal retinular cells (Figs. 2 and 8, *cl. dst.*) are flattened, and applied to the sides of the cones. They measure about $70\,\mu$ in length and possess, in addition to their distal processes, shorter proximal ones, which extend backward to the outer ends of the proximal retinular cells. As before, the cytoplasm is largely filled with black pigment granules, which, however, are often more concentrated in the body of the cell than elsewhere.

It must be obvious from this brief description that in considering the photomechanical changes of the distal retinular cells two factors are to be kept distinct: first, the lengthening and the shortening of the cell body, and, secondly, the distal and the proximal migration of the cell as a whole. That these two elements are distinct can be seen from the fact that in certain insects they are related to each other in a way just the reverse of that which occurs in Palæmonetes; thus, in Lasiocampa, as figured by Exner ('91, Taf. IV. Figs. 28, 29), the distal cells are *short* in their *distal* position, and *long* in their *proximal* one.

The average length of the bodies of the distal retinular cells from the left eye of a given animal prepared in light was about 30 μ. The average length of the corresponding cells from the right eye of the same animal prepared in the dark was about 70 μ. In a series of preparations taken from animals of approximately the same size as that just described, the lengthening of the distal cells in the dark took place at a rate indicated in the first of the following tables.

In a second series the shortening of the cells under the influence of light was shown to take place as indicated in the second table.

The distal and the proximal migration of the cells are difficult to define, because they are accompanied by the lengthening and the shortening of the cells. Taking the nucleus as a fixed point in the cell, the maximum distance of migration is about 50 μ. In the migration from the proximal to the distal extreme, made in the dark, the cell traverses this distance in about two hours. The migration in the reverse direction, under the influence of light, is completed in about one hour and three quarters.

As the cells move outward, their distal processes shorten and their proximal ones form and lengthen. As they move inward, their distal ones elongate and their proximal ones shorten and finally disappear. The rates of these changes, as well as of those given in the preceding paragraphs, are indicated in the following tables of summaries, in which the varying lengths of the parts of the cells are given for successive periods.

Migration in the Distal Direction (in the Dark).

Time.	0 hr.	¼ hr.	½ hr.	¾ hr.	1 hr.	1¼ hr.	1½ hr.	1¾ hr.	2 hr.
Length of distal process .	130[1]	125	110	95	85	65	55	40	30
Length of cell body . . .	30	35	50	55	60	70	70	70	70
Length of proximal process	0	0	0	10	15	25	35	50	60

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[1] All measurements of length are expressed in mikra (thousandths of a millimeter).

Migration in the Proximal Direction (in the Light).

Time.	0 hr.	¼ hr.	½ hr.	¾ hr.	1 hr.	1¼ hr.	1½ hr.	1¾ hr.	2 hr.
Length of distal process .	30	35	55	80	100	115	125	130	130
Length of cell body . . .	70	70	65	60	50	40	35	30	30
Length of proximal process	60	55	40	20	10	5	0	0	0

The changes induced in the distal retinular cells by the light are completed, then, in a period between an hour and a half and an hour and three quarters long. The changes that take place in the dark require for their completion from an hour and three quarters to two hours.

Rough estimates of the time necessary for the completion of these changes in different arthropods have been made by various investigators. Szczawinska ('91, p. 552) states that in Astacus the condition characteristic for the dark is reached in six hours, that for the light in two hours. Exner ('91, p. 70) states that in an insect, Lasiocampa, the changes require about half an hour, and Kiesel ('94, p. 105) gives the same time for Plusia. Herrick ('91, p. 455) believes that in Palæmonetes the changes are accomplished in about twenty-five minutes, an estimate that I should regard as rather too low.

Exner ('91, p. 70) has suggested that muscle fibres might be concerned in the migration of the distal retinular cells, an idea that gains some support from the fact that in the eyes of some insects structures like muscle fibres have been seen and described. In the crustacean retina, however, Exner was unable to find anything like muscles. At first sight it might seem probable that what I have described as the proximal and distal processes of the distal retinular cells might be muscular in nature. But the facts that the proximal process disappears entirely during the proximal migration of the cell, and that the distal one seems never to be firmly attached near the periphery of the retina, are opposed to this view. Moreover, in the distal process, which, on the whole, is the more muscle-like of the two, I have been unable to discover any evidence of transverse enlargement in the shortened condition, such as a contracted muscle exhibits. The cell in its distal migration seems to move over the fibre rather than to be drawn onward by a contraction of the fibre. Further evidence against the muscular nature of the motor mechanism of these cells is to be found in the rate at which the movement takes place; 50 μ in two hours is exceptionally slow for the action of any kind of muscle. These observations have led me to conclude that muscular action, as ordinarily understood, has nothing to do with the migration of the distal retinular cells. Obviously, ciliary action is in no way connected with the movements of these cells, and there is left then only amœboid movement as a means of explaining these changes. Each distal cell might be compared to an amœba, which in its migrations outward and inward uses its processes to guide its general motion. The rate and general character of the movement agree well with this explanation. In one respect, however, there is disagreement. Herrick ('91, p. 455), in his account of the action of the distal cells in Palæmonetes, states that, on contracting, these cells fold together somewhat as a ribbon might be folded transversely to its length (cf. Fig. 10), and he believes that, on expanding, they unfold again. This condition is one not easily reconciled with amœboid movement.

Through the kindness of Professor Herrick I have had the privilege of studying his preparations, and I can confirm his statement that in the contracted condition (Figs. 9 and 10) the cells exhibit a series of transverse folds, which are entirely absent from the expanded form. These folds, however, occur, so far as I am aware, only in eyes which have been kept an exceptionally long time in the dark (thirty-eight days in the case of Professor Herrick's specimens), and are then exposed to the light. In my own preparations, none of which had been kept in the dark more than twelve hours, no trace of such folding could be discovered, and I have therefore been led to regard these folds as abnormalities induced by protracted retention in the dark. Notwithstanding this interpretation of the folds, they throw important light, I believe, upon the normal action of the distal retinular cells.

The exact form of these folds is not so simple as might at first be supposed. The body of each cell in its contracted condition consists of an elongated thickened axial portion and two lateral wing-like expansions, each of which terminates in a rather sharp edge. In other words, these distal cells, when contracted, instead of assuming the usual ovoid form, retain more or less the shape that they had when expanded (Fig. 8). In a longitudinal section through the axial portion of the body of one of these cells (Fig. 9) slight folds are observable. In similar sections through the edge of the lateral wings (Fig. 10) the folding is seen to be much more pronounced. The folds are most conspicuous at the edges of the wings, and lose in prominence toward the axial part of the cell. Another peculiarity of these folded cells, as compared with those kept a shorter time in the dark, is that they shorten only to about five sevenths of their original length instead of to three sevenths. Thus their long retention in the dark seems to have prevented a return to the more completely contracted condition normal for the light. The fact that the more peripheral parts of the cells in the contracted condition are the more wrinkled indicates that the axial part has retained its contractile nature more completely than the peripheral parts, and suggests the idea that, since this axis contracts in a definite direction, it must possess something of the nature of a muscular core. It seems to me probable that, whilst the periphery of these cells may be characterized by amœboid movements, the core acts in a more circumscribed way, much as a muscle would. If this is so, the distal and proximal migrations, as well as the expansion of the cell body, are probably manifestations of its amœboid movements, while its shortening is probably due in the main to the muscle-like contraction of its central core. Objection might be raised to this combination of different modes of motion, were it not generally admitted that muscular action is, after all, only a more circumscribed form of amœboid movement.

The presence of a contractile axis in the distal retinular cells is further rendered probable by the fact that in Mysis (Parker, '91, p. 120) an axial core free from pigment has been observed in each distal retinular cell. At the time I first noticed these cores I suspected that they might be the remains of nervous axes, but I now believe there are stronger reasons for suspecting them of being contractile bodies.

The following table gives by way of *summary* the periods required for the completion of the various photomechanical changes in the retina of Palæmonetes.

	From dark to light.	From light to dark.
Proximal retinular cells	½ hr. to ¾ hr.	¾ hr. to 1 hr.
Distal retinular cells	1½ hr. to 1¾ hr.	1¾ hr. to 2 hr.
Accessory pigment cells	¾ hr. to 1 hr.	1¾ hr. to 2 hr.

It is a noteworthy fact, that of these changes those that take place in the light (positive stimulus) are always accomplished more rapidly than the corresponding reversals in the dark. To this statement an apparent exception may be found in the tables on pages 285 and 286, in which are recorded the periods for the contraction and expansion of the distal retinular cells. The body of the distal cell contracts in the light in an interval between 90 and 105 minutes, and expands in the dark in between 60 and 75 minutes, thus apparently accomplishing a change more rapidly in the dark than in the light. The expansion and contraction are, however, not simple operations, but are complicated by the simultaneous production or absorption of the large proximal processes, and it is possible that the discrepancy just pointed out is to be accounted for by this complication.

Before leaving this subject I wish to call attention to the comparative slowness with which all the photomechanical changes of the retina, but particularly those of the distal retinular cells, take place. Exner has shown that the amount of effective light that enters the eye is, in all probability, largely controlled by the action of the distal cells, and has therefore called them the iris pigment. The slowness with which they respond, however, shows clearly that in their action they have little resemblance to the iris of the vertebrate eye, and that their changes correspond only to the more general changes in the amount of light in their surroundings. The name iris pigment seems to me, therefore, somewhat misleading, and hence I prefer to retain the name of distal retinular cells, which indicates at once the present position and the probable origin of these cells, namely, from cells that once formed a part of the retinula itself (Parker, '95, p. 64).

Sympathetic Photomechanical Changes.

To ascertain whether the retinas in the two eyes of Palæmonetes were sympathetic toward each other in the same sense that Engelmann believed the retinas in the eyes of vertebrates were, I carried out two sets of experiments, in both of which animals were so placed that one eye was in the dark while the other was exposed to the light. After a sufficient period both eyes were prepared and examined. The two sets of experiments differed only in that I used different means to accomplish the exposure. In one set I tied a living shrimp to the inside of a light-proof box, in which a small hole was made so as to allow one optic stalk of the animal to project into the lighted exterior. Care was taken that the small space between the optic stalk and the edge of the hole should be filled with an opaque material (a mixture of thick Canada balsam and lampblack). After several hours the animal was killed, and its eyes prepared. In the other set of experiments one optic stalk of a living animal was covered with a considerable quantity of the mixture of balsam and lampblack, and, after allowing the animal to swim in a brightly illuminated dish for several hours, it was killed, and both its eyes prepared and examined.

The two sets of experiments yielded essentially the same results, namely, the eyes exposed to the light always presented the condition normal for the light, and those kept in the dark always showed an approach, more or less incomplete, to the condition characteristic for the dark. This incompleteness might be taken as evidence of a partial sympathetic relation between the two retinas; but I believe it is to be explained otherwise. In both sets of experiments the eyes supposed to be blinded were in reality only partially cut off from the light. In the experiment with the light-proof box, I know by actual observation that more or less light made its way *through the optic stalk* that projected outward to the exterior, and thus gained access to the interior of the box. If this is true of the experiment with the box, it is very probable that in the second experiment light passed up through the base of the blinded stalk, and thus reached at least the proximal part of the retina.

These experiments, then, are not wholly conclusive, but, so far as they go, indicate considerable independence in the relations of the two optic stalks. For reasons to be given later, in connection with the experiments on excised stalks, I believe I am justified in concluding that the two retinas are, in reality, wholly independent of each other.

Localized Photomechanical Changes.

Another question that naturally presents itself is, whether different parts of the same retina are sympathetic toward one another, or whether they are entirely independent, i. e. whether or not a retina responds locally to stimulus.

To test this matter, I put minute drops of the mixture of balsam and lampblack on the corneal cuticula of the eyes of several shrimps, and let them swim for a few hours in well illuminated basins. On examining sections of their eyes later, it was found that under each mass of applied pigment the retinal cells showed a condition characteristic for the dark. This was most pronounced in the distal retinular cells, but was also observable in the proximal retinular cells, as well as in the accessory pigment cells. This experiment shows beyond a doubt that the elements of the retina act locally, and respond to differences of light and dark independently of one another. This independence furthermore explains what is not infrequently seen in sections of otherwise normal eyes that have been kept in the dark, namely, occasional single proximal retinular cells which, instead of having their pigment granules transported to the retinal fibres, still hold them in their bodies. Such cells have probably suffered some pathological change by which their individual photomechanical functions have been interfered with. This independence in the action of parts of the retina has already been affirmed by Exner ('91, p. 66) for the compound eyes of insects.

Photomechanical Changes in Excised Eyes and Retinas.

The extent to which the photomechanical changes in the retina are influenced by the central nervous organs has never been determined, I believe, for any arthropod. That some such influence is exerted is implied by several investigators; thus Stefanowska ('90, p. 156) states that, in preparing insects' eyes, she cut the heads of the animals in two so as to prevent the nervous centres from affecting the retinal pigment cells, and Szczawinska ('91, p. 531) recommends as a fixing reagent a hot solution of corrosive sublimate, because the action is so rapid that it is not necessary to use other means of intercepting the central nervous influences. This belief, that the central nervous organs can exert an influence on the retinal pigment cells, is not to my knowledge the result of direct experiment, but is the application to other groups of animals of a generalization first made by Engelmann and his followers for vertebrates. As is well known, Engelmann showed that, when one eye of a frog was protected from the light, the illumination of the other eye, or even of a portion of the surface of the body, sufficed to produce in the pigment cells of the protected eye a condition characteristic for the light. This observation naturally led to the conclusion that the pigment cells of the retina were controlled in their movements by the central nervous organs, and that the optic nerve transmitted impulses centrifugally as well as centripetally. Fick ('95, pp. 77 and 81), however, has recently demonstrated that the same changes occur in a frog's eye even after the optic nerve and sympathetic nerves have been cut, and that therefore the central nervous organs take no part in these changes.

Before turning to the experimental evidence obtained from Palæmonetes, it will be well to consider some of the consequences of this question. In order that the central nervous organs should have any influence on the retinal pigment cells, the two sets of structures must be in nervous connection. So far as is known, the only structures in the retina of

Palæmonetes that have nervous connections are the proximal retinular cells, the accessory cells and the distal cells not being supplied with nerves. Since photomechanical changes occur in both the accessory and the distal cells, the inference might be drawn that in these instances the changes were necessarily independent of the central nervous organs. But it might also be argued that these very changes indicate nervous connections that have escaped the eye of the anatomist. To this it might be replied that, as the nervous connections of the proximal cells are so very obvious, it is highly improbable that the distal and accessory cells have a hitherto undescribed nerve supply. So far, then, as the purely anatomical relations are concerned, they indicate that the photomechanical changes, in the accessory and distal cells at least, are independent of central nervous influences.

In the case of the proximal retinular cells, where each cell possesses a single nerve fibre, the central nervous organs might control the pigment changes. However, if they do, the retinal fibres afford, so far as I know, the first good instance of normal double conduction. Since each retinal nerve fibre is the one nervous process from some proximal retinular cell, and since all these cells show photomechanical changes, it follows that, if these changes are controlled by the central nervous organs, all retinal fibres must transmit central impulses peripherad. As these same fibres are the only nervous connections between the retina and the central nervous organs, some at least must also transmit retinal impulses centrad. Therefore, if it can be shown that the central nervous organs influence the photomechanical changes in the proximal retinular cells, it is likewise demonstrated that double conduction is a natural occurrence. As Fick ('95, p. 73) justly remarks, the solution of this problem involves one of the most fundamental principles concerning the transmission of nervous impulses.

The method by which I proceeded to test this matter in Palæmonetes consisted in examining the changes that went on in eyes after their connection with the central nervous organs had been severed. These connections were conveniently cut in one of two places; either the whole optic stalk was excised, in which case the optic nerve was cut between the brain and the optic ganglia, leaving the latter in normal connection with the retina, or the retinal end of the stalk was cut off, thus separating the retina in the region of the retinal nerve fibres from the optic ganglia as well as from the brain.

To ascertain whether the brain had any influence over the retinal pigment, the following experiments were tried. Four live shrimps, whose eyes were in the condition characteristic for the dark, were decapitated; their right optic stalks were cut off and put in a light moist chamber, and their left stalks, likewise cut off, were placed, as a check on the results of the experiment, in a dark moist chamber. After an interval of two hours both sets of stalks were hardened and afterwards cut and examined. In a corresponding way optic stalks in the condition characteristic for the light were cut off and subjected to the dark.

The results of these experiments are shown in the following tables.

Four Right Optic Stalks in Dark Condition cut off and placed in the Light two Hours.

	Complete change.	Partial change.	No change.
Proximal retinular cells . . .	0	3	1
Distal retinular cells	0	3	1
Accessory cells	0	2	2

The four left optic stalks cut from the same animals and retained, as a check, in a dark chamber, all presented on examination the condition typical for the dark.

Four Right Optic Stalks in Light Condition cut off and placed in the Dark two Hours.

	Complete change.	Partial change.	No change.
Proximal retinular cells . . .	1	3	0
Distal retinular cells	1	3	0
Accessory cells	1	1	2

The four left optic stalks cut from the same animals and retained, as a check, in a light chamber, all presented on examination the condition typical for the light.

It is obvious from these observations that, after the excision of an optic stalk, the photomechanical changes may still take place, if not completely, at least partially, and it might be inferred from this that the brain exerted at least a partial influence over these changes. This conclusion, however, is invalidated by the fact that in one case recorded in the second table the photomechanical changes were carried out *completely*, thus demonstrating that the brain is not in any way essential to these changes. Why this completeness was not seen in other cases I am unable to state positively, though I believe it was owing to the changes that gradually appear in the tissue of the stalk after its severance from the body of the animal. When an optic stalk is excised, the blood in it soon coagulates and other alterations doubtless start up, which finally result in the complete death of the tissues of the stalk. It is these alterations, I believe, that overtake and bring to a standstill the slowly progressing photomechanical movements. But, whatever may be the true explanation of the incompleteness of the changes in excised stalks, the general conclusion remains unaffected, that in Palæmonetes the brain is not essential to the photomechanical changes in the retina.

This conclusion has an important bearing on the question of the sympathetic relations of the two retinas in a given animal. Since the two retinas are nervously connected only through the brain, and since the retinas are not influenced from the brain, it follows that the two retinas cannot be sympathetically related, a conclusion to which observations already recorded have likewise pointed.

If the photomechanical changes are not dependent in any degree on the brain, it may still be asked whether they are not influenced by the optic ganglia. To answer this question, I carried out on excised retinas a series of experiments similar to those just described for the optic stalks. It is much more difficult to separate the retina from the optic ganglia than it is to separate the optic stalk from the brain, but with careful manipulation it can be done, and the following tables give the results of experiments carried out upon such retinas.

Four Right Retinas in Dark Condition cut off and placed in the Light about two Hours.

	Complete change.	Partial change.	No change.
Proximal retinular cells . . .	0	4	0
Distal retinular cells	0	3	1
Accessory cells	0	4	0

The four left retinas kept in the dark as checks on the experiment exhibited the normal condition for the dark.

Four Right Retinas in Light Condition cut off and placed in the Dark about two Hours.

	Complete change.	Partial change.	No change.
Proximal retinular cells . . .	0	2	2
Distal retinular cells	0	4	0
Accessory cells	0	3	1

The four right retinas kept in the light as checks on the experiment exhibited the normal condition for the light.

Although, as the tables show, no case of excised retina with complete photomechanical changes has been observed, several of the cases were so nearly complete that I have no hesitancy in stating that, in my opinion, the photomechanical changes in the retina are as little influenced by the optic ganglia as by the brain.

These experiments, then, lead to two conclusions: first, the brain of Palæmonetes is not essential to the complete photomechanical changes of the retinal pigment cells; and, secondly, the optic ganglia are likewise unessential to these changes. In the latter case, however, the possibility of a slight influence must be admitted. The photomechanical changes of the retinal pigment cells are, in my opinion, induced by the direct influence of the presence or absence of light on these cells. Each cell, then, so far as its mode of action is concerned, is not comparable to a muscle controlled by an efferent nerve, but to a more or less independent organism, which receives a direct stimulus from the exterior, and responds appropriately. The uniformity usually shown by the photomechanical movements in the retina as a whole is to be understood as an individual but uniform reaction of many separate elements to a uniform stimulus. There is nothing in the action of the retinal pigment cells of Palæmonetes that supports the idea of normal double conduction of nervous impulses.

General Summary.

1. The only parts of the retina in Palæmonetes that exhibit photomechanical changes are the three kinds of pigment cells.

2. The proximal retinular cells contain black pigment granules. *In the light* these are scattered more or less uniformly throughout the whole

length of the cell, including the retinal nerve fibre. There are slight concentrations of pigment at the distal end of the cell and around the rhabdome. *In the dark* the pigment is limited to the retinal nerve fibres.

3. The change from the dark condition to the light one is accomplished in from 30 to 45 minutes. The reverse change requires 45 to 60 minutes.

4. These changes are probably due to internal protoplasmic movements, by which the pigment granules in the cells are moved in one or other direction.

5. The accesssory pigment cells contain a yellowish white pigment. *In the light* this is massed partly in the base of the retina, and partly near the distal surface of the first optic ganglion. The two pigment masses are connected by pigmented strands. *In the dark* the pigment is almost entirely in the base of the retina.

6. The change from the dark condition to the light one is accomplished in from 45 to 60 minutes; the reverse change, in from 105 to 120 minutes.

7. These changes are probably produced by amœboid movements of the cells.

8. The distal retinular cells contain black pigment granules. *In the light* they are contracted, and occupy a proximal position in the retina surrounding the axis of the ommatidium near the outer ends of the proximal retinular cells. *In the dark* they are expanded (flattened), and occupy a distal position in the retina, surrounding more or less completely the sides of the cone.

9. The change from the dark condition to the light one is accomplished in from 90 to 105 minutes; the reverse change requires from 105 to 120 minutes.

10. These changes are produced in part by an amœboid movement of the cell, and probably in part by a muscle-like contraction of its axial portion.

11. Each set of photomechanical changes carried out in the light is completed in less time than the corresponding set of reverse changes carried out in the dark.

12. The photomechanical condition of the retina in one eye has no effect upon that in the other eye; i. e. the retinas are not sympathetic.

13. The photomechanical action within the retina is localized, small groups of pigment cells responding to local stimulation.

14. In excised eyes (optic nerve cut), complete photomechanical changes may occur, thus proving that the brain is not essential to these changes.

15. In excised retinas (retinal nerve fibres cut), nearly complete photomechanical changes may occur, thus showing that the optic ganglia are probably not essential to these changes.

16. The incompleteness of the changes in either the excised eyes or excised retinas is probably due to the death of the retinal tissues before the photomechanical changes have been completed.

17. The three kinds of retinal pigment cells probably respond to direct stimulation from without, and are not influenced by nervous impulses from within. There is no good evidence in favor of normal double conduction of nervous impulses.

NOTE.

Since the preceding pages were written, Rosenstadt's ('96) paper on the structure of the compound eyes in Decapods has been published. This contains a brief general account of the migration of the retinal pigment in these crustaceans, and calls for a word of comment. In his description of the directions of motion shown by the pigment under various conditions of light, Rosenstadt agrees with Exner and later investigators, but in his account of how this movement is accomplished he stands entirely alone. His conception of the process can best be put in his own words (Rosenstadt, '96, p. 759): "Beim Uebergange des Lichtauges in ein Dunkelauge gehen mit dem Pigmente volgende Veränderungen vor sich: Das Pigment tritt aus dem vorderen Ende der Retinulazellen [= proximal retinular cells] und wohl auch aus den Retinapigmentzellen [= rudimentary retinular cells] aus. Dasselbe wird von den Fortsätzen der Irispigmentzellen [= distal retinular cells] aufgenommen, die, wie wir gesehen haben, mit dem im Vorderende der Retinulae angesammelten Pigmente im Contact stehen. An diesen Fortsätzen kriecht nun das Pigment hinauf; es findet eine Art Pigmentinfiltration der Irispigmentzellen statt. Gleichzeitig wandert das Pigment nach hinten zu aus den Retinulazellen aus und gelangt hinter die Membrana fenestrata [= basement membrane], wo es von den mit Ausläufern versehenen Zellen aufgenommen wird." This idea that the pigment migrates from one cell to another is, so far as Rosenstadt's account goes, entirely unsupported by direct evidence, and seems to me an unwarranted assumption. The proximal movement of the pigment from the distal end of the retinula to the opposite side of the basement membrane is certainly accomplished within the limits of one set of cells, for, as I have shown in this paper, the pigment even when entirely proximal to the basement membrane lies in the thick retinal nerve fibres, which are merely processes from the proximal retinular cells. Although it cannot be stated with certainty that there is no exchange of pigment between the distal and the proximal retinular cells in Palæmonetes, for in this crustacean in bright light these two kinds of cells are closely applied to each other, it is perfectly certain that in other decapods, as for instance Palæmon, no such exchange is possible; for, as Exner ('91, Taf. V. Fig. 51) has shown, and I can confirm his observations, the pigmented parts of the distal and the proximal retinular cells never touch, even under full light. These reasons, together with the facts set down in the present paper, confirm me in the belief that Rosenstadt's explanation of the migration of the pigment is erroneous, and that the one presented in the foregoing account is correct.

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EXPLANATION OF PLATE.

All the figures were taken from preparations of the eyes of *Palæmonetes vulgaris* Stimp. They were drawn with the aid of an Abbé camera, and are all magnified 335 diameters.

ABBREVIATIONS.

cl. con.	Cone cell.	*fbr. r.*	Retinal nerve fibre.
cl. dst.	Distal retinular cell.	*mb. ba.*	Basement membrane.
cl. px.	Proximal retinular cell.	*nl. con.*	Nucleus of cone cell.
cl. sn.	Accessory pigment cell.	*nl. crn.*	Nucleus of corneal hypodermis cell.
con.	Cone.	*nl. px.*	Nucleus of proximal retinular cell.
crn.	Corneal cuticula.	*rhb.*	Rhabdome.

Fig. 1. Longitudinal section of an ommatidium, showing the arrangement of pigment characteristic for the light.

Fig. 2. Longitudinal section of an ommatidium, showing the arrangement of pigment characteristic for the dark.

Fig. 3. Transverse section of a cone from an ommatidium, such as is shown in Fig. 2 (dark).

Fig. 4. Transverse section through the proximal processes of the distal retinular cells in an ommatidium such as that shown in Fig. 2 (dark).

Fig. 5. Transverse section through the distal retinular cells of an ommatidium such as that shown in Fig. 1 (light).

Fig. 6. Transverse section through the retinula (rhabdome and proximal retinular cells) of an ommatidium like that shown in Fig. 1 (light).

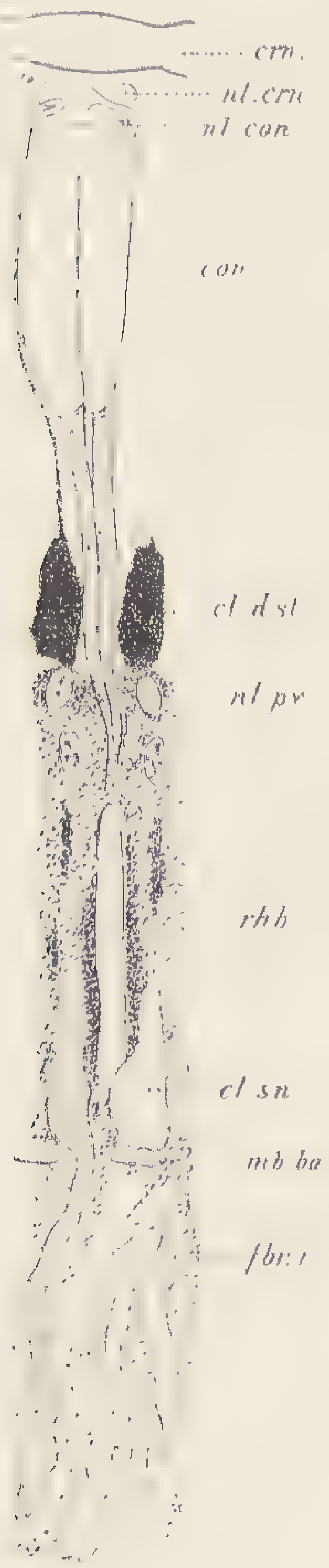
Fig. 7. Transverse section through two groups of retinal nerve fibres.

Fig. 8. Lateral view of a cone with one of its two distal retinular cells still attached. The distal retinular cell shows the condition characteristic for the dark. The preparation was isolated from a retina macerated in Müller's fluid.

Fig. 9. Longitudinal section through the bodies of two distal retinular cells, which show slight foldings accompanying their shortening. The preparation was made from an animal which had been kept in the dark thirty-eight days and then exposed to light for four hours and three quarters. The figure was drawn from preparations made by Professor F. H. Herrick, who kindly granted the author the privilege of studying them and making drawings from them.

Fig. 10. Longitudinal section through the edges of two distal retinular cells (see p. 287), from the same set of sections as that from which Fig. 9 was drawn.

1.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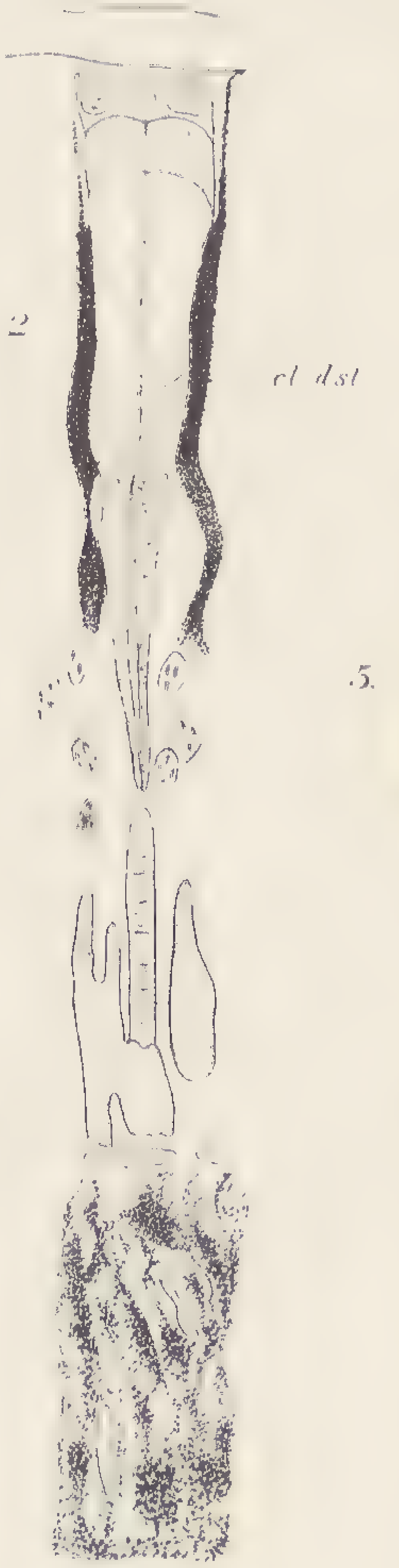

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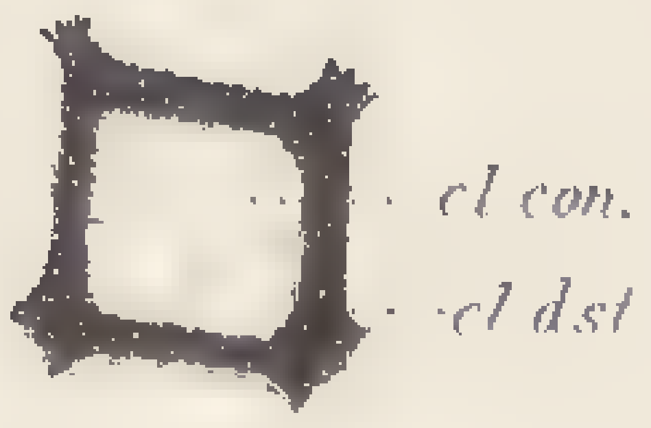
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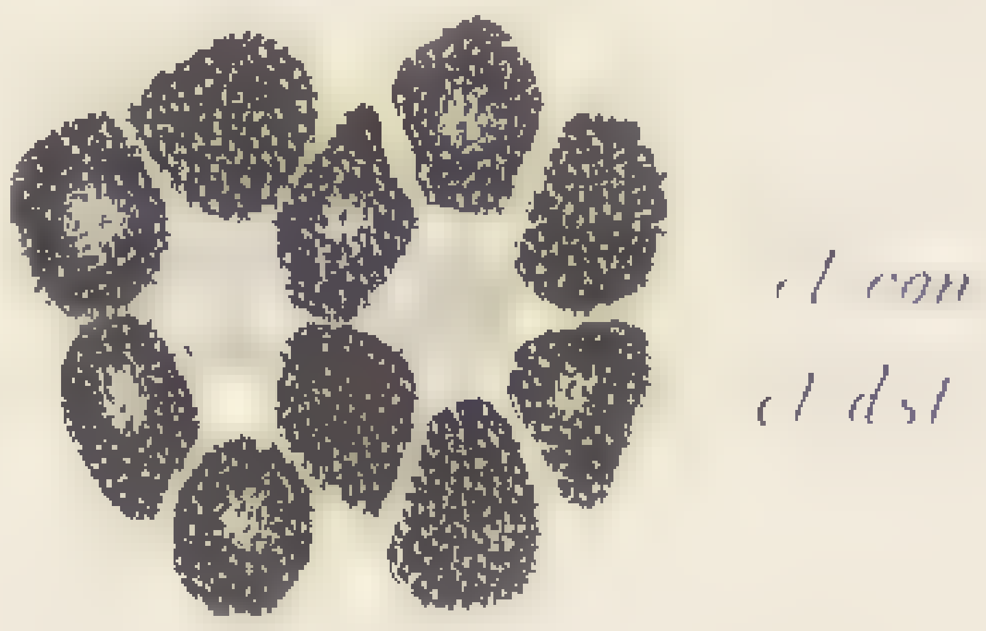
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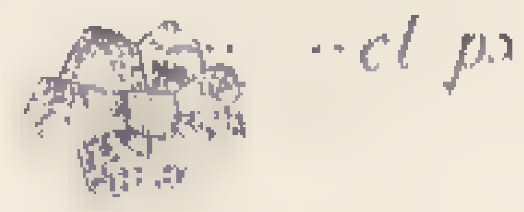

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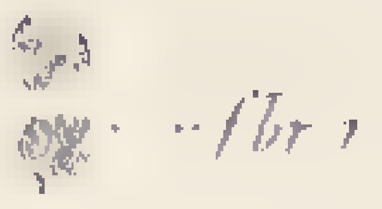

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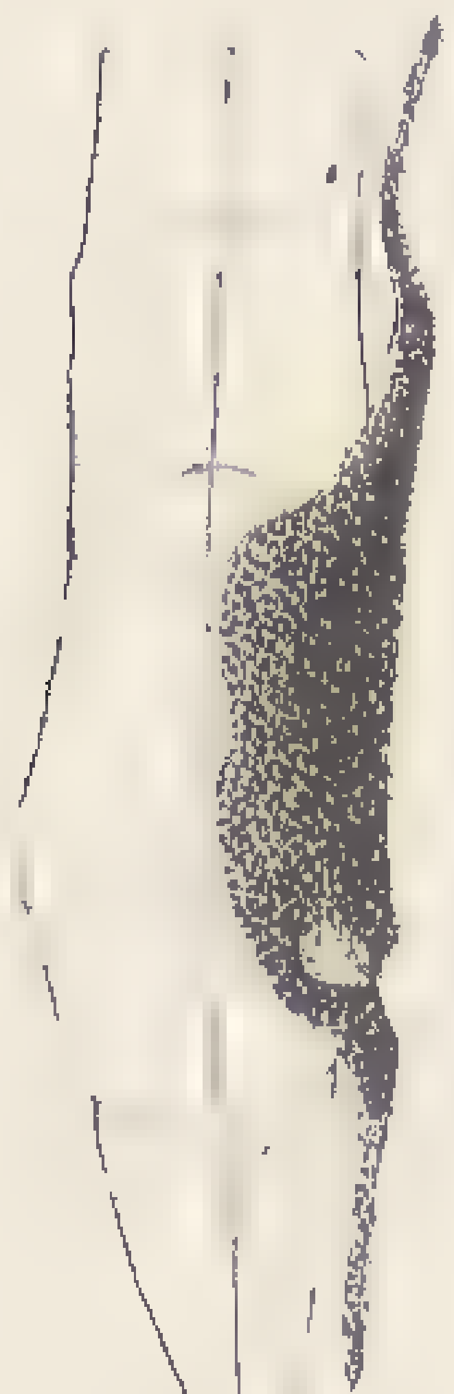

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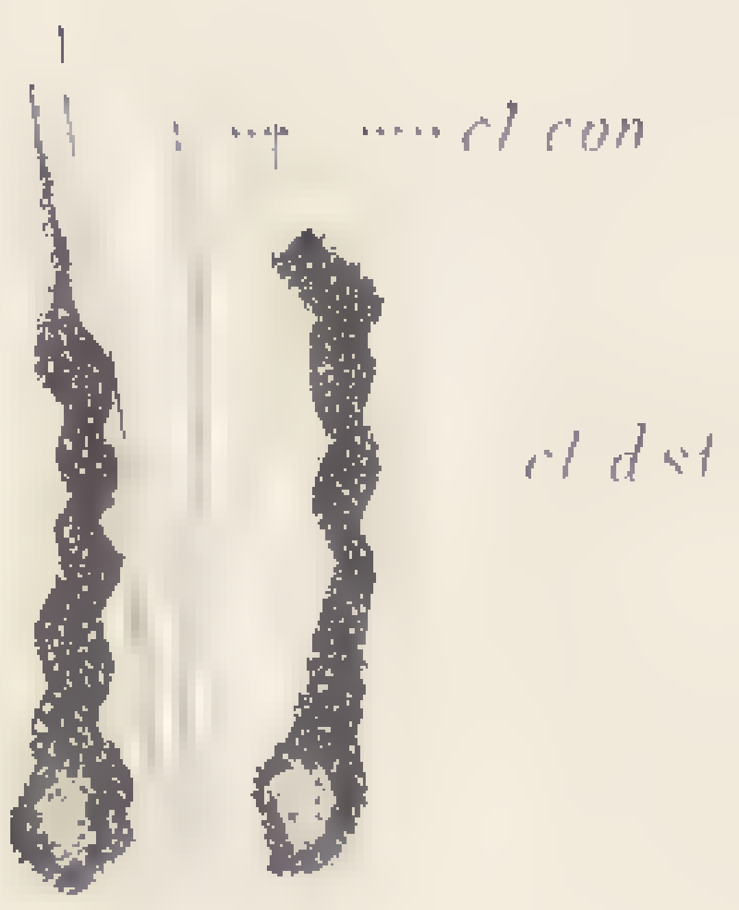
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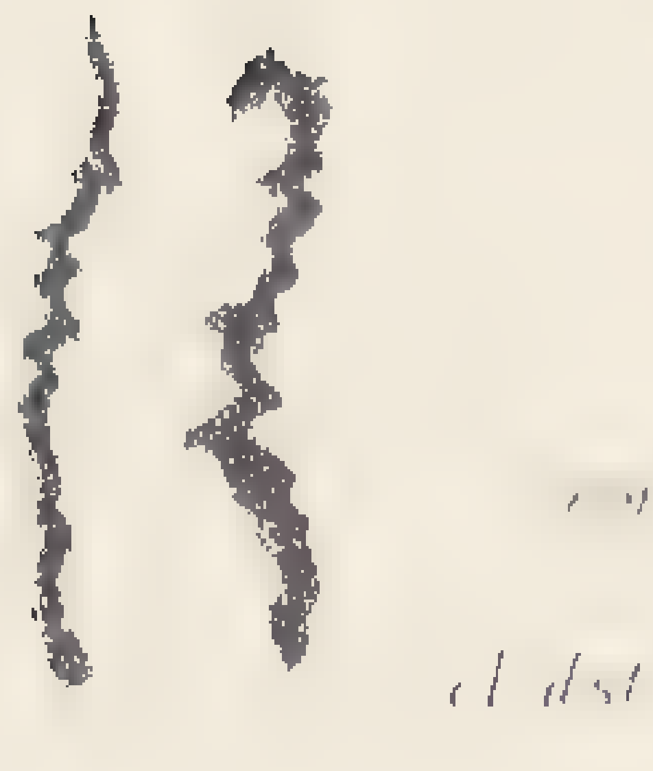

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